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2017 SPRING MEETING OF THE WPSA UK BRANCH

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- 15 J. E. MARTIN, K. CHRISTENSEN, Y. VIZZIER-THAXTON, M. A. MITCHELL, AND D. E. F. MCKEEGAN. A new method of stunning poultry: evaluation of physiological and behavioural responses to Low Atmospheric Pressure Stunning (LAPS) in broilers.
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PROOF ONLY

Reduction of prevalence, numbers, virulence and antibiotic resistance of foodborne pathogens in commercial broiler caeca samples

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APPLICATION

Prevalence, numbers, virulence and antibiotic resistance of foodborne pathogens can be reduced by the inclusion of Original XPC in poultry diets.

in accordance with governmental regulations and company management standards.

INTRODUCTION

Antibiotic usage in animal production has led to concerns in public, regulatory and scientific arenas about resistant bacteria in farm animals and its transfer to humans. Research has shown that livestock may serve as a reservoir of resistant bacteria that may transfer to our food system, some of zoonotic concern such as *Salmonella*, *Escherichia coli* and *Campylobacter*, and subsequently decrease the effectiveness of the antibiotic compounds. Interventions are needed to reduce the reservoir of resistance genes in food animals.

RESULTS

Feeding TRT to broilers significantly reduced ($P < 0.0001$) *Salmonella* prevalence compared to CON (8.6% vs. 23.5%, respectively). A significant reduction ($P < 0.0001$) was also observed for *Salmonella* numbers in TRT fed birds vs. CON (11.5 vs. 150.8 colony-forming unit (CFU)/g, respectively). *Salmonella* virulence was significantly lowered ($P < 0.0001$) in isolates from TRT birds compared to CON (0.17% vs. 1.05%, respectively), representing a 6-fold reduction in virulence. *Salmonella* antibiotic resistance was significantly lowered ($P < 0.0001$) in isolates from TRT birds compared to CON (florfenicol: 2.02% vs. 12.54%; ceftiofur: 0.78% vs. 9.43%; enrofloxacin: 0.01% vs. 3.87%, respectively). *E. coli* resistance to ceftiofur was significantly lowered ($P < 0.0001$) compared to CON (27.17% vs. 67.15%, respectively).

MATERIALS AND METHODS

Two field studies were conducted to determine the effects of feeding Original XPC (Diamond V, Cedar Rapids, IA, USA), a broad-spectrum immune modulator, on reducing *Salmonella* and *E. coli* in broilers, including an evaluation of *Salmonella* prevalence, numbers, virulence and antibiotic resistance to ceftiofur, florfenicol and enrofloxacin and *E. coli* resistance to ceftiofur. In the *Salmonella* study, a total of 116 commercial broiler flocks from 8 companies were monitored. In the *E. coli* study, a total of 64 commercial flocks from 5 broiler companies were monitored. In both studies, houses were fed either a diet that contained 1.25 kg/MT of the Original XPC (TRT) or the company standard diet (CON). Depending on the company protocol, at processing, one caecum was collected from between 50 and 100 birds per flock (4075 and 2700 total caecum for the *Salmonella* and *E. coli* study, respectively) during evisceration at the processing plant. Caeca samples were shipped overnight to the lab, where they were analysed for *Salmonella* prevalence and numbers including measurement of virulence and antibiotic resistance. *E. coli* samples were tested for antibiotic resistance to ceftiofur. Data were analysed in SAS using either the chi-square procedure or Generalised Linear Modelling with feeding treatment as the main effect. Birds within each company were reared in accordance to each company's management standards and bird welfare practices. Birds were humanely processed

CONCLUSION

The addition of TRT was associated with a significant reduction in *Salmonella* prevalence. *Salmonella* recovered from TRT birds were 6 times less likely to cause illness in a downstream host as measured by an *in vitro* invasion assay. This reduction in virulence has been associated with a measured reduction in the expression of the gene *hlyA*, the global regulator of *Salmonella* virulence (Feye *et al.* 2016). *E. coli* are more likely to be susceptible to the antibiotic ceftiofur. These data suggest that the addition of TRT to the diet can be an effective intervention on the farm level to reduce *Salmonella* in broilers. The antibiotic resistance data also suggest that TRT can be a successful strategy in reducing antimicrobial resistance in poultry production.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the commercial operations that helped with caeca sampling.

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Effect of dietary phytonutrients in ameliorating coccidiosis challenge

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APPLICATION

Vaccination reduced feed efficiency at an early stage that was later compensated. Dietary betaine improved overall feed efficiency and no interactions between treatments were observed.

INTRODUCTION

Concerns over the future use of anticoccidials have increased the need to better understand the effects of coccidiosis infection on bird growth and response to feed additives (Amerah and Ravindran 2015). The poultry industry also needs an alternative to anticoccidials used so far. A beneficial effect of some phytonutrients, e.g. betaine, on reducing performance losses of broilers exposed to coccidiosis has been reported (Amerah and Ravindran 2015). Feeding fenugreek seeds (*Trigonella foenum-graecum*) also improved growth performance of broilers but the impact on protecting birds from cocci infection has not been studied (Adil *et al.* 2015). The aim of the study was to examine the effect of betaine and sotolon (extract from fenugreek) supplementation on bird growth of broiler chickens fed proprietary broiler feed and exposed to experimental coccidia challenge. Gut perturbation was assessed by measuring of excessive intestinal fluid (IF), gizzard erosion (GIZ) and foamy caecal content (FCC) following standard procedures.

MATERIAL AND METHODS

The trial was approved by the Animal Experimental Committee of Harper Adams University. Day-old Ross 308 male chicks were obtained from a commercial hatchery, weighed and assigned to 48 pens (5 birds per pen) situated in 4 separated rooms (24 pens in a room). Birds were fed 1 of 4 mash diets; a control (C) wheat-soybean diet contained per kg 210 g crude protein (CP) and 12.95 MJ metabolisable energy (ME); C + betaine (1.0 g/kg feed) (Danisco Animal Nutrition, Wiltshire, UK); C + sotolon (0.2 g/kg feed) (4,5-Dimethyl-3-hydroxy-2,5-dihydrofuran-2-one; Sigma-Aldrich Co.); C + betaine (1.0 g/kg

feed) + sotolon (0.2 g/kg feed). The birds in two of the rooms were given a live coccidiosis challenge (Paracox 5; MSD Animal Health, UK), providing 8 experimental treatments in total. The environment in the rooms followed industry recommendations. The weight of the birds and feed intake were recorded on days 0, 7, 14 and 21. At the end of the study, one bird per pen was dissected and gut perturbation was assessed. Performance data were analysed by split-plot analysis of variance (ANOVA). Gut health data were analysed using Pearson chi-square test to identify significant differences between treatments.

RESULTS

Vaccination increased ($P < 0.05$) feed conversion ratio (FCR) from 0 to 14 d of age, although there were no differences ($P > 0.05$) at 21 d of age (Table). Betaine supplementation reduced ($P < 0.05$) the overall FCR from 0 to 21 d of age. The overall weight gain (WG) of the birds was not affected ($P > 0.05$) by the experimental treatments. Dietary sotolon supplementation did not have ($P > 0.05$) an impact on the studied variables. Increased FCC was observed in challenged birds ($P = 0.001$). Overall, the challenged birds had numerically ($P > 0.05$) higher IF and GIZ compared to the non-challenged. No interaction ($P > 0.05$) between coccidiosis challenged and phytonutrients was observed on any of the studied variables.

CONCLUSION

It can be concluded that coccidiosis challenge increased FCR during early growth but its effect disappeared later. The challenge increased overall gut perturbation. Dietary betaine reduced overall FCR.

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Table. Effect of coccidia challenge and betaine and sotolon supplementation on the weight gain (g/day/bird), feed intake (g/day/bird) and feed conversion ratio (FCR, g/g) in broilers fed a wheat-soy based diet (1 to 21 d post-hatch)

Parameter	Challenged				Betaine				Sotolon			
	No	Yes	SEM	P	No	Yes	SEM	P	No	Yes	SEM	P
FI 0–7	22.0	22.0	1.048	NS	22.1	21.9	1.039	NS	21.1	22.9	1.039	NS
FI 0–14	34.01	35.01	0.211	0.081	34.9	34.3	0.685	NS	34.4	34.8	0.685	NS
FI 0–21	49.2	49.9	0.220	NS	49.9	49.2	0.570	NS	48.9	50.2	0.570	NS
WG 0–7	13.8	14.3	0.249	NS	14.0	14.0	0.283	NS	14.01	13.9	0.283	NS
WG 0–14	23.0	22.8	0.073	NS	22.6	23.2	0.365	NS	23.2	22.6	0.365	NS
WG 0–21	30.5	30.8	0.171	NS	30.3	31.1	0.398	NS	30.6	30.7	0.398	NS
FCR 0–7	1.602	1.572	0.0884	NS	1.605	1.570	0.0777	NS	1.524	1.651	0.0777	NS
FCR 0–14	1.468	1.636	0.0108	0.047	1.536	1.469	0.0289	NS	1.487	1.517	0.0289	NS
FCR 0–21	1.612	1.613	0.0085	NS	1.644	1.582	0.0207	0.043	1.602	1.624	0.0207	NS

225 The mechanisms underlying the beneficial effects of precision delivery coated butyrate in a necrotic enteritis model in broilers

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230 APPLICATION

Precision delivery of a coated butyrate product administered to necrotic enteritis (NE) challenged and control broilers can improve performance via changes in gut health parameters, modulation of the immune system, and changes in hormone production.

INTRODUCTION

NE is an important enteric disease in poultry, caused by the overgrowth of certain *Clostridium perfringens* strains, which evokes intestinal tissue damage. Aiming to test non-antibiotic alternative strategies to counter the negative consequences of subclinical NE on performance and gut health, we set up experiments to evaluate the effects of butyrate in NE-challenged broilers. Butyrate is known to trigger several physiological responses associated with improved gut health (Guilloteau *et al.* 2010). Moreover, the product under evaluation (ADIMIX Precision, "AP", Nutriad, Belgium) was precision delivery coated; this means that butyrate is well protected from complete gastric absorption, and will be delivered directly to the luminal side of the intestine, where it is hypothesised to be most effective in supporting gut integrity.

MATERIALS AND METHODS

800 Male Arbor Acres broilers were allocated to 4 treatment groups, each consisting of 10 replicates of 20 birds. The experiment was set up using a 2 × 2 factorial design: birds were supplemented with AP or not and were *Clostridium*-challenged ("Clos") or not. Birds supplemented with AP were given the following inclusion levels in their feed: in starter feed (day 01–14): 1.00 kg/T; in grower feed (day 15–28): 0.50 kg/T; in finisher feed (day 29–35): 0.25 kg/T. All the broilers were vaccinated against ND (days 7 and 21) and IB (day 14). NE-challenged broilers were infected with a *Clostridium perfringens* type A B2 NET B strain, associated with NE. The bacteria were orally administered at a dose of 10⁸ CFU/bird/day, for 4 days (days 14–17). At days 21 and 28, 10 birds per group were necropsied and examined for

pathological lesions of the small intestine. Lesions were evaluated according to the system of Shojadoost *et al.* (2012) and given a value ranging from 0 (no gross lesions observed) to 6 (diffuse necrosis). Live weight, feed intake and FCR were monitored for the entire period of the experiment. Serum antibody responses to vaccination against Newcastle Disease antigen were determined via a hemagglutination inhibition (HI) assay (day 35). RNA was extracted from liver tissue of ten 35-day-old birds/treatment and qPCR analysed IGF-1 expression, relative to b-actin expression. Statistical test was done using one-way ANOVA with post-hoc Duncan's multiple range test, with significance set at $P < 0.05$. The experiment was carried out according to the National regulations on animal welfare and approved by the Institutional Animal Ethical Committee (Cairo, Egypt).

RESULTS

The results of the analyses are summarised in the Table.

CONCLUSION

Both in the challenged and in unchallenged groups, AP-fed birds outperformed the corresponding control groups. The necrotic lesion scores suggest that these results are at least partially explained by the effect of AP on prevention or repair of intestinal tissue damage. In addition, AP-supplemented birds expressed more IGF-1, which is reported to be related to body growth. The relative bursa weight was significantly increased in challenged birds that were administered AP. The HI titer against ND viral antigens were reduced in NE-challenged birds; on the contrary, birds that received AP had significantly higher titers, which is suggestive for an AP-induced boost of immune responses.

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Table. Performance and analytical results from the 4 different treatments

	No Clos.; no AP	No Clos.; AP	Clos.; No AP	Clos.; AP
Final weight (g)	1863 ^b	1916 ^a	1823 ^c	1852 ^b
FCR	1.77	1.74	1.81	1.78
No. of birds with lesion score 3-2-1-0 (day 21) [#]	0-0-0-10	0-0-0-10	6-4-0-0	3-2-5-0
No. of birds with lesion score 3-2-1-0 (day 28) [#]	0-0-0-10	0-0-0-10	0-2-3-5	0-0-4-6
Hepatic IGF-1 expr.	1.69 ^a	1.78 ^a	1.12 ^c	1.43 ^b
Bursa weight (% of total weight)	0.20 ^b	0.22 ^b	0.21 ^b	0.27 ^a
HI titer against ND vaccine (day 35)	5.44 ^b	7.20 ^a	3.50 ^c	6.80 ^a

Different superscript alphabets in a single row denote $P < 0.05$.

[#]No birds were seen with a score higher than 3.

Efficacy of TYPLEX™ chelate in broilers with natural *Campylobacter* challenge

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APPLICATION

Reducing *Campylobacter* carriage in poultry is challenging but essential to control this major cause of human bacterial gastroenteritis worldwide. Data presented here show the efficacy of a novel feed additive to reduce *Campylobacter* carriage.

INTRODUCTION

It is estimated that 50–80% of human campylobacteriosis may be attributed to the chicken reservoir (EFSA, 2010). TYPLEX™ chelate (ferric tyrosine [FeTyr₃], Akeso Biomedical, Inc.) may prevent binding of *Campylobacter* to the gut wall and thus reduce campylobacteriosis. This study investigated the efficacy of ferric tyrosine on growth performance and *Campylobacter* carriage in broilers exposed to litter that was naturally contaminated with *Campylobacter jejuni*.

MATERIALS AND METHODS

Four hundred and eighty 1-d old male broilers (Ross 308) were allocated to 6 dietary treatments in a randomised block design, each having 8 replicate pens of 10 birds per pen. The iso-nitrogenous and iso-energetic wheat-soyabean-meal-based control diet (T1) was manufactured as one batch and 5 treatments (T2–T6) were generated by addition of ferric tyrosine at 0.01, 0.02, 0.05, 0.10 and 0.20 g/kg feed, respectively. At day 20, litter from a commercial flock that had tested positive for *Campylobacter* was added to all pens (2 kg/pen). Cloacal swabs from one bird/pen were taken at day 18. At day 42, all birds were euthanised and pooled caeca per pen were used for *Campylobacter* enumeration. The growth performance and

log₁₀ transformed *Campylobacter* counts were evaluated by analysis of variance. Significant ($P < 0.05$) means were separated using Tukey, supplemented with contrasts to compare control vs ferric tyrosine diets, and linear regression over different feed content of ferric tyrosine. The study was approved by the animal ethics committee prior to start.

RESULTS

Feed content of ferric tyrosine was confirmed using a red microtracer marker and all values were within the expected range. The overall growth performance data (0–42 d) showed that birds in ferric tyrosine supplemented groups per se had 4.0% greater body weight gain (BWG) than control birds. However, the differences were only significant ($P < 0.05$) when T5 and T6 were compared with T1 (Table). Since feed intake was similar ($P > 0.05$) between treatments, this growth improvement concurred with improved FCR values in birds fed ferric tyrosine. The European Production Efficiency Factor (EPEF) was 8.9% higher than controls in birds fed ferric tyrosine. All cloacal swabs taken prior to challenge were negative for *Campylobacter*. The caecal *Campylobacter* counts at 42 d were highest in controls compared to ferric-tyrosine-fed birds per se. However, the pair-wise differences indicated that this arose largely from significant differences between the control group and those fed 0.05 and 0.20 g ferric tyrosine/kg feed. The dosage effect of ferric tyrosine was linear ($P < 0.05$) for BWG, FCR, EPEF and *Campylobacter* counts. The combination of linear regression and Tukey multiple comparisons suggests that optimal inclusion to minimise *Campylobacter* carriage was 0.05 g ferric tyrosine/kg feed. However, weight gain and EPEF data showed further improvement when dosage was increased from 0.05 to 0.10 g ferric tyrosine/kg feed.

Table. The overall growth response, European Production Efficiency Factor (EPEF) and *Campylobacter* counts in caeca of broilers naturally challenged with *Campylobacter* over the global trial period (0–42d)

Treatment	Weight gain	Feed Intake		<i>Campylobacter</i>	
	(kg/bird)	(kg/bird)	FCR	EPEF	log ₁₀ CFU/g
T1: Natural challenge, no FeTyr ₃	3.305 ^a	5.358	1.637 ^b	467.2 ^a	5.124 ^b
T2: Natural challenge + 0.01 g FeTyr ₃ /kg feed	3.442 ^{ab}	5.295	1.546 ^a	498.7 ^{ab}	4.716 ^{ab}
T3: Natural challenge + 0.02 g FeTyr ₃ /kg feed	3.425 ^{ab}	5.306	1.550 ^a	520.0 ^{ab}	4.692 ^{ab}
T4: Natural challenge + 0.05 g FeTyr ₃ /kg feed	3.358 ^{ab}	5.205	1.550 ^a	489.6 ^{ab}	4.377 ^a
T5: Natural challenge + 0.10 g FeTyr ₃ /kg feed	3.490 ^b	5.348	1.543 ^a	513.9 ^{ab}	4.64 ^{ab}
T6: Natural challenge + 0.20 g FeTyr ₃ /kg feed	3.492 ^b	5.345	1.536 ^a	528.4 ^b	4.472 ^a
P-value	0.007	0.480	<.001	0.016	0.007
Standard error of deviation (SED)	0.053	0.083	0.019	17.59	0.201
Control vs. FeTyr ₃ (T1 vs T2, T3, T4, T5 and T6)	0.002	0.378	<.001	0.003	<.001
Linear	0.003	0.989	<.001	0.004	0.002

FCR = Mortality adjusted feed to gain ratio;

different superscript alphabets within a same column indicate significant differences at $P < 0.05$.

CONCLUSION

This study demonstrates that ferric tyrosine significantly improved growth performance and reduced the carriage of *Campylobacter* compared to the unsupplemented control in broilers naturally challenged with *Campylobacter*.

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FUNDING

The authors acknowledge the funding provided by Akeso Biomedical, Inc., Waltham, MA for this study.

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The potential application of insects for poultry nutrition

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INTRODUCTION

The use of insects to bioconvert organic matter into feed grade protein has received significant attention over the last 4 years. In addition to protein, products of this insect production may have potential application in poultry. Rearing insects on biological materials that are not part of the feed or food chain offers an opportunity to effectively capture nutrient resources as insect biomass. Insects naturally form a significant part of the diet of wild birds. Methods of farming and processing insects for use in animal feed are being developed. The use of insects in non-food-producing livestock (i.e. pets) is allowed, but currently the use of processed insects in feed for farmed livestock is highly restricted through regulation.

A major review of insects in feed was published by EFSA in 2015, and in the light of this, it is anticipated that insect protein will be permitted in aqua feed from July 2017. The input materials used in the production of insects for feed will be tightly controlled to avoid any risks of contamination. It is anticipated that processed insect protein will be permitted in poultry feed within the European Union in a further 3–4 years.

The primary interest for insect producers is the production of a dried, defatted, high-quality protein material, typically of 50–60% crude protein. Currently, insect meal demands a price similar to fishmeal and is being used in some pet food diets. There will be a significant opportunity for insect protein in aqua feed where use of fishmeal is common place for carnivorous species. Research is supporting the use of insect meal in poultry diets as a substitute for conventional protein sources. However, commercial uptake of insect meal in poultry diets would require lower costs of insect protein than are currently in the market. Driven by a growing demand for use in aquafeed, investment and development in the insect meal production over the coming years will bring economies of scale and improved technical and logistical efficiency. This will allow insect meal to become cost competitive with conventional protein sources in poultry diets.

The use of insect oil, a by-product of the protein meal, is permitted under EU law and is currently being evaluated by at least one feed company in The Netherlands. The composition of insect oil will vary with the species of insect, the stage of harvest and the substrate material. Manipulating the fatty acid profile may have benefits. One characteristic of particular interest is that the oil of the Black Soldier Fly larvae, one of the insect species targeted for feed production, contains a high concentration of lauric

acid. Lauric acid is a 12-carbon fatty acid with activity against gram-positive bacteria which could result in extra calorific benefits from the insect fat.

Supplementation of diets with live larvae is also being investigated on aspects of bird behaviour, performance and health. More scientific study will be required to investigate the live insect effect, the reasons for it and the practical application of any positive outcomes to the commercial situation.

Larvae, as a means of self-preservation, secrete antimicrobial peptides called defensins. These defensin peptides are being investigated for their potential application as antimicrobials in poultry. Separation of the chitin or chitosan from the protein meal may also prove beneficial. Removal would further enhance the protein value of the meal itself. Additionally, purified chitin extract may offer opportunities as a functional ingredient in poultry as well as in wider agricultural uses.

Clearly, opportunities exist for insects as a future livestock feed material. The short- to medium-term potential for insect meal in feed appears to be greatest for pet and aqua diets. The use of insect oils in poultry feed offers a more immediate opportunity. Feeding of live insects and the use of functional insect-derived products warrant further investigation.

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Future prospects for gene editing of poultry genomes

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INTRODUCTION

The chicken is used widely as a model organism for vertebrate developmental biology. It is unique amongst animal models in that it is also a livestock animal of major economic importance, crucial for food security in the developed and developing world as well as a substantial reservoir of zoonotic diseases. The rapid advances in genomic information and genetic technologies in the chick have created major opportunities, but their exploitation is constrained by lack of methods to efficiently

investigate and modify gene function in poultry. The advent of site-specific nucleases, zinc finger nucleases, TALE (transcriptional activator-like effector) nucleases (TALENs) and Crispr/CAS9 (clustered regularly interspaced short palindromic repeat) vectors have revolutionised and accelerated our ability to selectively modify the genome of farm animals. In this presentation, we will present the current state of art and future potentials for "gene editing" of poultry; producing targeted, specific modifications of the chicken genome using these genetic tools.

A new method of stunning poultry: evaluation of physiological and behavioural responses to Low Atmospheric Pressure Stunning (LAPS) in broilers

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APPLICATION

Presentation of a novel stunning method for poultry, which is consistent and similar to welfare assessment results with controlled atmosphere stunning with inert gases.

electroencephalogram (EEG) and electrocardiogram (ECG) responses to LAPS in 170 broilers (Cobb 500 male broilers), and interpreted their welfare impact. Trial 1: characterised the responses of broilers exposed to LAPS in 30 triplets at two temperature settings (TS3 (13–18°C); TS4 (5–12°C)). Trial 2: examined the influence of illumination and sham treatment in a 2 × 2 factorial design (20 pairs per treatment), at TS4 only. In each triplet/pair, one bird was instrumented for continuous recording of EEG and ECG, and the behaviour of all birds was recorded by digital video recorder. All data were summarised in Microsoft Excel (2010) spreadsheets and analysed using Genstat (14th edition) and using Generalised Linear Mixed Models.

INTRODUCTION

In 2015, over 59 billion broiler chickens were produced globally for human consumption, and therefore, the method of how these birds are stunned and slaughtered is paramount to maintaining welfare on a large scale. A novel pre-slaughter stunning method for chickens has been developed, where birds are rendered unconscious by progressive hypobaric hypoxia, this approach shares many of the welfare advantages of controlled atmosphere stunning systems, which irreversibly stun poultry by exposure to hypoxic and/or hypercapnic gas mixtures. Low Atmospheric Pressure Stunning (LAPS) involves gradual decompression (280s) according to a prescribed curve.

RESULTS

In both trials, birds showed a consistent sequence of behaviours during LAPS (ataxia, loss of posture (LOP), clonic convulsions and motionless) (Fig.). TS4 showed shorter LOP latencies than TS3 ($P < 0.001$), but in Trial 2, illumination had no effect ($P = 0.250$). During LAPS, EEG spectral analysis revealed progressive decreases in median frequency and increases in total power (PTOT), followed by decreases in PTOT before the onset of an isoelectric state (brain death). TS had no effect on latency to ($F50 < 6.8\text{Hz}$ – general anaesthetic plane/unconscious), but illumination and sham did (LAPS/dark = 39.1 ± 6.3 s; LAPS/light = 53.6 ± 11.8 s; sham/dark = 12.8 ± 5.2 s; sham/light = 88.0 ± 29.5 s ($P < 0.001$)). Illumination increased activity and dark induced sleep, but slow-wave EEG was seen in both. ECG showed latency to pronounced

MATERIALS AND METHODS

The work was conducted at the University of Arkansas (United States) and the experiments were specifically authorised by the University of Arkansas Institutional Animal Care and Use Committee (Protocol 15 031). The LAPS system was developed by Technocatch LLC in Mississippi, USA, and the pressure curves applied by the process are patented. We examined behavioural,



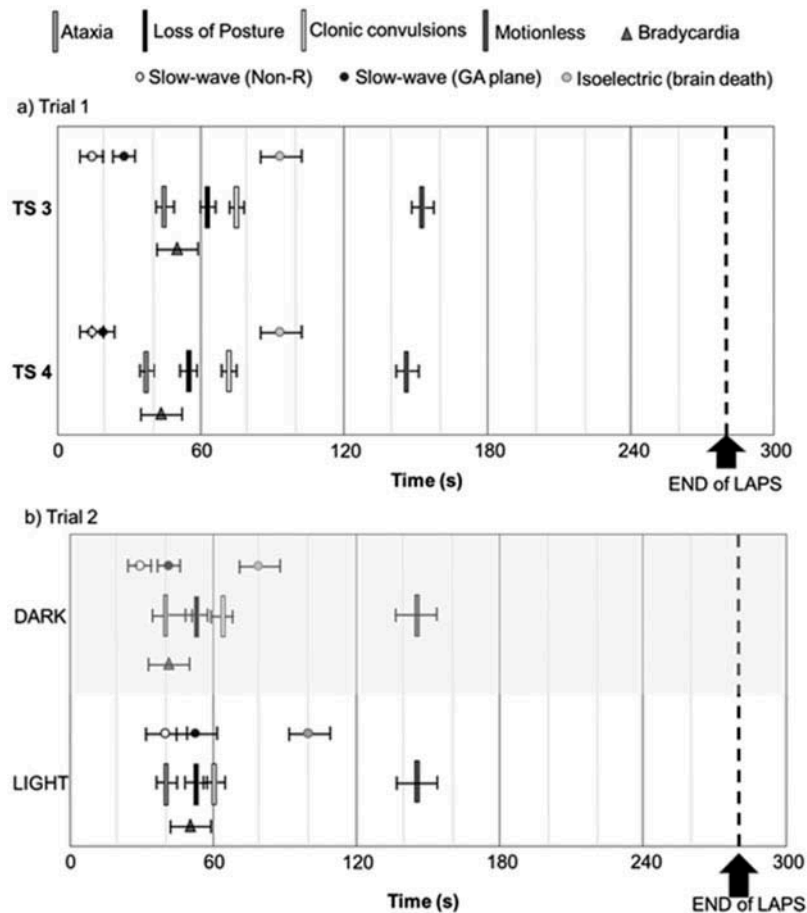


Fig. Mean (\pm SE) latencies for key behaviour, EEG and ECG measures for Trial 1 (a); and Trial 2 (b).

bradycardia in LAPS was affected by TS ($P = 0.021$). In Trial 2, bradycardia was absent in sham and was not affected by illumination.

CONCLUSION

These results suggest that responses to LAPS are consistent and similar to those observed with controlled atmosphere stunning with inert gases, therefore providing evidence of a humane alternative method for stunning poultry.

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An investigation into the effects of residual feed intake and growth rate on measures linked to leg bone health in broiler chickens

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APPLICATION

Increased growth rate (GR) was associated with reduced tibial ash content; however residual feed intake (RFI) did not appear to have a significant effect on measures of leg bone structure and strength in broilers.

INTRODUCTION

Olkowski *et al.* (2011) suggested that insufficiency of bone matrix proteins in fast-growing broilers may reduce mineralisation of bone tissue (due to an inadequate protein skeleton for normal mineral propagation). It is suggested that this results in bone tissue that is marked for pathological re-modelling, and which is characterised by reduced protein content in the bone organic matrix, and reduced trabecular bone. These changes may predispose chickens to conditions such as femoral head necrosis. Theoretically, feed-use efficiency may also affect these bone characteristics in a manner that is independent of GR, as it is linked with altered protein turnover rates in other species. This study examined the effects of growth rate (GR) and feed efficiency (measured as RFI) on measures of leg bone structure, strength and composition in broiler chickens, and on markers of bone remodelling.

MATERIALS AND METHODS

Fifty-three mixed-sex Cobb 500FF chickens were used over three replicate batches. Bird weight was recorded weekly from 7 days of age until slaughter at 42–43 days, and feed intake was recorded. RFI was calculated for the interval between 7 and 38 days. Within each batch and gender, birds with the lowest (best), medium and highest RFI values were selected (17, 20 and 16, in total, respectively). At slaughter, both legs were removed and stored at -20°C . Following thawing, the right tibia was dissected out and morphology and weight were recorded. The marrow was removed, and the bone was dried and ground to a fine powder before near infra-red spectroscopy (NIRS) analysis of protein content. The breaking strength of the left tibia was determined using a 3-point bending to failure test. These bones were then broken up and dried at 100°C for 24 h to determine dry matter, and placed in a furnace at 600°C for 24 h to determine ash. The trabecular architecture of the “neck” of the right femur from 23 birds in total (representing extremes of GR within RFI groups) was assessed using micro-CT scanning. Serum collected at slaughter was analysed for alkaline phosphatase and osteocalcin. Behavioural scans were conducted at 5-min intervals over two 17-h light periods between 36 and 40 days to determine level of standing. RFI, BW at day 42 and GR were compared between RFI groups using one-way ANOVA in SPSS (version 21). The effects of RFI and GR on bone parameters were analysed using a GLM with RFI group as a fixed factor, GR as a covariate and batch as a random factor. Where residuals were not normally

distributed, the relationship between GR and bone/behaviour parameters was assessed using a Spearman's rank correlation and the effect of RFI group examined using Kruskal-Wallis tests. This study was performed under the Animals (Scientific Procedures) Act, 1986.

RESULTS

BW, GR and activity levels did not differ between RFI groups ($P > 0.05$), but RFI values differed significantly ((mean (\pm SE)) low RFI = $-231.85 (26.88)^{\text{a}}$, medium RFI = $-4.23 (11.67)^{\text{b}}$, high RFI = $231.87 (34.57)^{\text{c}}$, $P < 0.01$). There was no difference between RFI groups in any of the measures of bone structure, strength, composition (e.g. protein content) or remodelling ($P > 0.05$). As expected, there was a positive association between GR and measures of tibia weight, length and width (Table). GR was negatively associated with % ash, but not related to breaking strength (Table). Bone volume and volume fraction measures from micro-CT analysis were positively associated with GR ($P < 0.05$). A positive correlation was also shown between GR and the proportion of behavioural scans where the bird was sitting ($r = 0.332$, $P < 0.05$).

CONCLUSION

RFI did not significantly influence bone structure, strength, composition and remodelling measures used. Faster GR was associated with reduced tibial ash content and with reduced behavioural activity; however, these effects did not translate into adverse effects on breaking strength and bone microstructure. In addition, effects of GR on the ash content of tibias did not appear to be accompanied by changes to bone protein content or to markers of bone turnover.

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Table. Estimated marginal means of tibia morphology and composition for RFI groups and estimate for effects of GR

	Low RFI	Aver. RFI	High RFI	P	GR estimate	P
Weight (g)	20.70	20.42	19.55	NS	0.326	<0.001
Length (cm)	11.45	11.40	11.37	NS	0.032	<0.001
Width at centre (mm)	8.984	9.068	8.966	NS	0.057	<0.001
Ash (%)	37.70	37.11	36.99	NS	-0.111	<0.001
Breaking strength (N/mm ²)	3.68	3.80	3.82	NS	-0.008	NS

Basal hypothalamic orexigenic gene expression is sexually dimorphic after re-feeding with normal and diluted diets

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APPLICATION

To potentially reduce the need for quantitative food restriction in broiler breeders, we need to understand the mechanisms controlling satiety and long-term BW control in birds. In this study, we have demonstrated that gut fill alone does not reduce the activity of neurones that increase food intake (orexigenic) in the brain of chickens. However, the orexigenic neurones in males are more active than in females, suggesting a role in determining more rapid male growth.

INTRODUCTION

We have demonstrated that the orexigenic genes, *ACRP* and *NPY*, in neurones of the basal hypothalamus brain region of female broiler breeder chickens are very sensitive to feeding history (Dunn *et al.* 2015, Dunn *et al.* 2013), distinguishing between short- and longer-term feed restriction and the timing of re-feeding. Expression of *POMC* and *CART* genes that encode peptides that inhibit feeding are less responsive. In broiler breeders, food restriction improves reproductive performance and general health but increases hunger. One approach to tackle the paradox is diluted diets. The aim of the experiment was a comparison of gene expression in the basal hypothalamus to distinguish in male and female broiler breeders between the mechanical effects of gut fullness induced by diluted diets and nutrition-related feedback signals resulting from food ingestion. Despite well-known differences in the *GR* of male and female chickens (Maniatis *et al.* 2013), we had no expectation of differences in the expression of orexigenic and anorectic genes between the sexes.

MATERIALS AND METHODS

The effect of re-feeding restricted-fed 12-week-old male and female broiler breeders ($N = 9-13$) on ad libitum (AL) diet for 2 days was compared with re-feeding a diet diluted with a non-nutritive bulking agent, ispaghula husk (IH). A control group remained on the standard restricted diet. The birds were reared on the restriction recommended in the Ross 308 management manual (2013) and the lighting schedule also followed these recommendations. IH absorbs water and results in a very large bulking action when ingested. The diet dilution was achieved by adding 15% IH by weight to the standard restricted diet ration. This was found from pilot studies to result in consumption of the ration over the entire light phase, whereas the normal restricted diet was consumed in a very short period after presentation. Birds were individually housed for accurate

estimation of food intake and the experiment was carried out in three replicated batches. To confirm the observed sex difference effects, the experiment was repeated using only AL and restricted broiler breeders ($N = 8$), and observations were also made in untreated male and female chickens of an unrelated breed. At the end of the experiment, the basal hypothalamus was dissected and frozen and gene expression of *ACRP*, *NPY*, *POMC* and *CART* was measured using real-time PCR. ANOVA (Genstat 13) was used for statistical analysis with transformation where appropriate, least significant difference was used to test difference between means if necessary. The study was carried out under project licence 7007909, and individual experiments were additionally approved by the Institute Ethics Committee.

RESULTS

IH-fed birds had a significantly increased crop content weight of 62 g compared to 15 g in the standard restricted birds and 87 g in AL birds. *ACRP* and *NPY* expression measured by real-time PCR was significantly decreased in birds that re-fed AL ($P < 0.001$) but was high and indistinguishable between restricted controls and birds that re-fed the IH diet. An inverse pattern was observed for *POMC* and *CART* expression, with expression being significantly higher in the AL re-fed group ($P < 0.001$). Interestingly, pronounced sex differences were observed in gene expression. *ACRP* ($P < 0.05$) and *NPY* ($P < 0.05$) mRNA levels were generally significantly higher in males compared to females within treatment groups, and this sex difference was larger within restricted control and IH groups than in the AL group. This effect was confirmed when the experiment was repeated using only restricted and AL groups, with the *ACRP* expression being higher in males than females ($P < 0.01$). In genetically distinct chickens, higher *ACRP* mRNA expression was observed in AL-fed males compared to females showing that the sex difference is not restricted to broilers.

CONCLUSION

Feeding a non-nutritive diet to induce prolonged crop and gut fill but did not reduce orexigenic gene expression after re-feeding. Expression of orexigenic genes in particular is significantly higher in males than females. Similar results were observed in a genetically distinct line of chickens that were not food restricted. The cause of this difference in gene expression in the hypothalamus between sexes is unknown but it may be either related to, or potentially drive, the faster *GR* of male chickens.

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Pancreatic peptide YY (PYY) expression is responsive to short-term nutritional state and the pancreas constitutes the major site of PYY mRNA expression in chickens

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APPLICATION

805 Welfare concerns surround hunger in broiler breeders because of the necessity to quantitatively restrict feed intake in order to maintain reproductive viability and health – the so-called “broiler-breeder paradox”. Optimising poultry management practices towards ameliorating food restriction in an economical and sustainable way depends on developing a better understanding of the molecular bases of hunger and satiety in domestic fowl. Here, we have demonstrated the response of the satiety hormone peptide YY (PYY) in the pancreas to food intake for the first time in birds.

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INTRODUCTION

As its name suggests, it is well known that pancreatic polypeptide (PP) is primarily produced in the pancreas, and constitutes the major pancreatic member of the neuropeptide Y family of hormones in vertebrates. The other members of the family – neuropeptide Y (NPY) and peptide YY (PYY) – are believed to be mainly expressed in the brain and gut, respectively. All three hormones are important factors affecting appetite and energy balance; PP and PYY are released peripherally to signal satiety to the brain via neuromodulatory (vagus) and neuroendocrine (arcuate nucleus) action, whereas NPY has a functionally opposite effect within the brain. Whilst chicken PP and NPY have been relatively well characterised, the gene sequence for chicken PYY has only recently been elucidated. Here, we show that the pancreas is the major site of PYY mRNA production in chickens, adding to the growing mammalian evidence that PYY expressed in the pancreas has an important functional role. We also present evidence that pancreatic PYY expression is regulated dependent on short-term satiety state in chickens, implicating it as a short-term satiety factor, whereas PP expression is not under short-term regulation.

MATERIALS AND METHODS

840 To initially investigate tissue distribution of PYY expression in chickens, four brown egg-laying hens were killed at peak of lay, and tissue samples from basal hypothalamus, peripheral

organs and several sections of the gastrointestinal tract were collected. To test the effect of short-term hunger as well as the distribution of expression in the pancreas, previously AL-fed broiler-layer hybrid chicks (age 14 d) were fasted for 3.5 h and then either re-fed AL or fasted for a further 7-8h before cull and collection of pancreas head and tail samples. These two treatment groups were balanced for sex and family ($N = 12$). RNA was isolated from samples homogenised in Trizol (Invitrogen) using the Direct-zol RNA miniprep kit (Zymo Research). Reverse transcription and qPCR were carried out as previously described (Whenham *et al.* 2015). All qPCR results were normalised to YWHAZ and/or LBR reference genes. All animal experiments were approved by the Roslin Institute Animal Welfare and Ethics Committee and carried out under UK Home Office project licence 70/7909.

RESULTS

Pancreatic (pan) PYY expression was higher than in any other tested tissue in AL laying hens, with the next-highest region of expression being the mid-proximal ileum (mpil) ($N = 4$; pan 4.64 ± 0.76 vs. mpil 1 ± 0.27 units, ANOVA, $P = 0.004$). Comparative *in situ* hybridisation of PYY mRNA in fixed sections from the two tissue types corroborated this result. Chicks that fed AL expressed 45% more pancreatic PYY than those fasted for 11 h ($F_{1,41} = 15.47$, $P < 0.001$), but no significant differences in PYY expression were detected between groups ($F_{1,41} = 0.68$, $P = 0.413$) and expression of neither gene differed between head and tail of the same pancreas.

CONCLUSION

These findings implicate the pancreas as a major source of circulating PYY. Further study is needed to ascertain the mechanism(s) of action of pancreatic PYY as compared to ileal PYY as tissue-differential modes of neuroactive signalling is feasible and might determine the effective dose at receptive sites *in vivo*. Taken together, data from feeding studies suggest that pancreatic PYY expression is responsive to feeding and acts as a short-term satiety factor. Further work to investigate how the expression and release of PYY is controlled in response to food intake and specific nutrient uptake may help us further understand growth in poultry and how it might be controlled.

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Differences in the feed conversion efficiency of broilers are not reflected in their liver metabolome

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APPLICATION

Analysis of the liver metabolome does not act as a biomarker for differences in the performance efficiency of growing broilers, suggesting that such differences are the result of differences in nutrient absorption rather than metabolism.

INTRODUCTION

Variations in FCR between seemingly similar bird flocks are costly for the poultry production industry. In the absence of overt disease, it can be difficult to ascertain the reason behind such differences, when birds are of the same genotype, are fed the same diet and are maintained in apparently the same environment. It would seem likely that any differences in the utilisation of feed between these birds would be as a result of differences in either the absorption of nutrients from the gut, or in the metabolism of those nutrients. Differences in nutrient metabolism would result in a difference in the liver metabolome, and this might provide an indicator of a better or worse performing bird. In this regard, the liver metabolome of the healthy chicken *Gallus gallus* has been reported (Le Roy *et al.* 2016), and the hypothesis in this study was that the metabolome of the healthy bird could be further differentiated to identify differences between the metabolism of birds that performed differently when they were fed the same diet and were kept in the same environment. The objective of this study was therefore to determine what differences there were in the liver metabolome of broiler chickens of similar weights which were of similar genotype, fed the same diet, but which had shown different FCR during their growth.

were weighed and FCR was calculated. Pens were ranked by FCR for the period 10–15 d, and again for the period 22–29 d. The three pens with the highest FCR and the three pens with the lowest FCR were selected for each time period. One bird from each pen was removed and humanely slaughtered at days 15 and 29. A liver sample was removed, snap frozen in liquid nitrogen and stored at –80°C pending analysis. Liver samples taken from birds from the selected pens were then extracted with methanol and analysed using ¹H NMR. Peak area and frequency from CPMG spectra were processed by principal component analysis (PCA) and orthogonal projections to latent structures discriminant analysis (OPLS-DA) to analyse differences between high- and low-performing birds. The effect of performance classification (high and low FCR) on bird weight and FCR was determined using a 2-sample *t*-test where significance was denoted as *P* < 0.05.

RESULTS

The FCR and liveweight of the two groups of birds at the two time intervals are summarised in the Table. There was no difference in bird liveweight, suggesting that at each time period, the birds from the two groups were of a similar physiological (and chronological) age. However, there was a significant difference in FCR. Despite this difference in the FCR of birds, the PCA analyses of liver metabolome spectra indicated no sample clustering, indicating no discrete differences in metabolome between high- and low-performing birds. This was further confirmed by OPLS-DA, since *R*²*Y* values (0.858, day 15; 0.846, day 29) gave indications of a good predictive model, while low *Q*² values (day 15 –0.715, day 29

MATERIALS AND METHODS

A total of 192 as hatched Ross 308 broiler chicks were randomly allocated as day-old chicks to one of 12 pens (16 chicks per pen). All pens were bedded with sawdust and were surrounded by a plastic sheet to exclude draughts. All birds were fed the same diet, which was a starter diet from days 1–10, then a grower/finisher diet from days 11–29. At days 10, 15, 22 and 29, birds and feed

Table. Effect of performance classification on bird performance

	High FCR	Low FCR	SEM	<i>P</i> -value
<i>Bird age 15 d</i>				
FCR	1.38	1.28	0.008	0.019
Bird liveweight (g)	333	342	7.6	0.021
<i>Bird age 29 d</i>				
FCR	1.62	1.52	0.008	0.571
Bird liveweight (g)	1234	1358	117.4	0.416

–0.404) and the related correlation plots indicated no effect of treatment at either point.

CONCLUSION

These results indicate that differences in the FCR of birds of a similar weight are not a consequence of differences in the liver metabolism of absorbed nutrients at either 15 or 29 d of age. Differences in FCR are therefore more

likely to be a result of differences in nutrient absorption, rather than differences in the metabolism of absorbed nutrients.

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Effect of supplementing broiler diets with bioavailable silica on tendon and bone strength

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APPLICATION

Bone breaking strength is significantly increased by supplementation with bioavailable silica which could have wide applications in mitigating lameness and skeletal damage across the poultry industry.

INTRODUCTION

In the past 50 years, the GR of broilers has increased by over 300% due to intense genetic selection, with birds reaching slaughter weight in half the time historically required (Waldenstedt 2006). Leg disorders are believed to be linked with this increased growth, and mortality with culls and downgrading of carcasses during processing accounting for 0.10–0.30 of total production losses. Silicon (Si) has been associated with calcification of bone and deficiencies have been linked to skeletal weaknesses (Carlisle 1972). More recently, studies supplementing broiler diets with bioavailable Si have shown increased tibial strength. However, the effect of Si on tendons has not been assessed, and it may be that the bone strength is a function of tendon musculo-skeletal effects. Therefore, the aim of this study was to compare the tendon and bone strength of birds supplemented with bioavailable Si with birds that fed a standard diet to determine whether there is a relationship between tendon and bone strength.

MATERIALS AND METHODS

576 Male Ross 308 broiler chicks were placed in 48 pens, with each pen holding 12 chicks and the facility divided into 24 four plots consisting of two pens. Twelve replicate plots were allocated to one of two treatments: either a standard diet (control), or an identical diet, supplemented with bioavailable silica at 1000 ppm (Silica). Both diets were standard wheat-soya-based commercial formulations for starter diets and fed AL as mash. On day 21, one bird per plot was euthanized and one femur, one tibia and one distal gastrocnemius tendon were collected. Tendons were stored frozen in 0.9% NaCl solution and all bones and

tendons were stored individually at –20°C until analysed. Tensile strength of the bones and tendons were analysed using a TA.XT plus texture analyser (Stable Microsystems, Guildford, UK) set up with a 50 kg load cell and a three point-bend fixture or tensile grips for the bones and tendons, respectively. Morphometric measurements were also taken of width, length, weight and cross-sectional diameters. Data was analysed via unpaired *t*-test and relationships assessed via Pearson Correlations using IBM SPSS version 23, with a statistical significance declared at $P < 0.05$.

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RESULTS

No significant difference ($P = 0.634$) was found between the tensile strength of tendons of the control birds and the Si-supplemented birds (Table), but there was a significant ($P < 0.001$) increase in both the femoral and tibial breaking strength in birds supplemented with Si (Table). A weak, negative correlation was found between tendon and bone strength on the control diet ($r = -0.288$), but no correlation was found between both the tibia and femur bones and the tendon strength of the birds supplemented with silica ($r = -0.038$) (Fig. 1 & 2).

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CONCLUSION

The results of this study provide a further indication that Si supplementation may be beneficial by increasing the bone strength, but there appears to be no impact of Si

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Table. Tendon and bone measures of birds at day 21 fed diet with and without 1000 ppm silica

Measure	Control	Si	P-value
Tendon strength/N	48.1 (4.97)	51.1 (3.90)	0.634
Time to breaking /s	12.9 (1.37)	14.46 (1.40)	0.419
Femur breaking strength /N	126.1 (8.88)	177.4 (6.15)	<0.001
Tibia breaking strength /N	134.1 (6.81)	174.5 (6.68)	<0.001

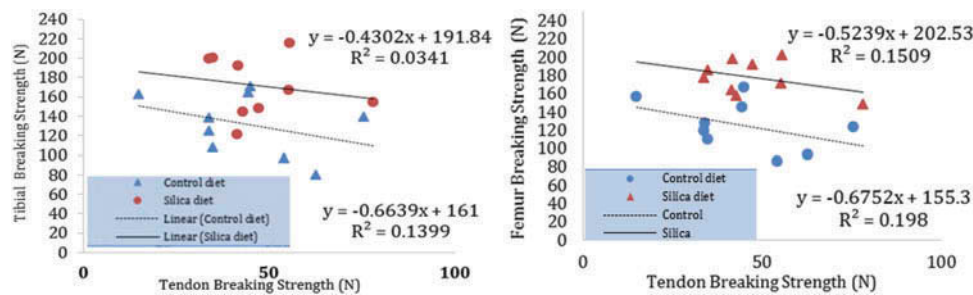


Fig. 1 & 2. The relationship between tibial and tendon breaking strength (Fig 1; see legend) and femoral and tendon strength (Fig 2; see legend).

supplementation on gastrocnemius tendon strength. The next step for this work is to relate bird performance to the parameters discussed here.

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Variability of metabolisable energy in different wheat cultivars for broilers

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APPLICATION

There were no differences in apparent ME of six current UK feed wheat cultivars.

INTRODUCTION

Wheat is often the only cereal used in broiler feed formulations in many countries, so its nutritional value and variations in feeding quality have large commercial importance (Ball et al., 2013). The nutritive quality of wheat is variable. Wheat is primarily used in broiler feeds for its content of available energy and there is considerable variation in the apparent metabolisable energy (AME) of different wheat samples. Thus, the prediction of AME is important. The aim of this study was to examine the differences in the chemical composition and AME of six UK feed wheat cultivars.

MATERIALS AND METHODS

All procedures were approved by The Animal Experimental Committee of Harper Adams University. Six UK wheat cultivars grown in 2015 on 4 sites at Yorkshire, Nottinghamshire, Cambridge, and Lincolnshire (17 samples in total) were selected to formulate 17 diets, including 670 g/kg of each wheat sample and 330 g/kg of balancer.

Three additional diets containing 470, 570 and 770 g/kg of one wheat sample (Santiago-Cambridge) were formulated to test linearity. The diets were made iso-nitrogenic by adding a mixture of starch and wheat protein isolate at the expense of wheat (25 g/kg). All diets were pelleted. Eight hundred male Ross broilers were allocated to 160 raised floor pens. Each diet was replicated 8 times, fed from 0 to 21 d age in a randomised complete block design. Excreta were quantitatively collected for the last three days and AME was determined following standard procedure. Data was statistically compared by randomised block ANOVA. Multiple linear regression analysis was used to assess the relationship between determined AME and the chemical composition of the wheat cultivars.

RESULTS

There were no differences between wheat cultivars in analysed starch, non-starch polysaccharides (NSP), the soluble proportion of the total NSP, ash, protein and determined AME. Samples grown at the Nottinghamshire site were significantly higher in protein ($P < 0.002$), ash ($P < 0.041$) and NSP ($P < 0.03$) from other samples and had numerically lower AME ($P = 0.120$). Stepwise multiple regression indicated that there was a significant relationship between combination of protein, ash, the soluble proportion of the total NSP and AME ($P < 0.05$; $r^2 = 0.57$; $SE\hat{Q} = 0.196$): $AME\ (MJ/kg\ DM) = 15.86\ (SE\ 0.701) - 0.01CP\ (SE\ 0.005) - 0.07Ash\ (SE\ 0.035) + 2.75solNSP\ proportion\ (SE\ 1.260)$.

Table. Chemical composition and apparent metabolisable energy (AME MJ/kg DM) of 17 wheat samples.

Wheat cultivars	Growing site	Chemical composition of wheat cultivars (g/kg DM)					AME
		Starch	NSP	Sol NSP	Protein	Ash	
Leeds	Nottinghamshire	678	98.2	0.1827	143	19.6	14.00
Leeds	Yorkshire	704	92.8	0.2294	117	16.0	14.25
Leeds	Lincolnshire	704	90.5	0.1794	127	15.3	13.94
Leeds	Cambridge	696	87.7	0.1569	116	12.8	14.44
KWS Santiago	Yorkshire	697	86.6	0.1749	117	15.9	14.38
KWS Santiago	Nottinghamshire	722	90.6	0.2032	138	17.9	13.82
KWS Santiago	Lincolnshire	671	80.1	0.1539	116	16.2	14.20
KWS Santiago	Cambridge	728	87.7	0.1282	123	18.0	13.68
KWS Lili	Yorkshire1	699	86.7	0.2167	122	16.3	14.03
KWS Lili	Yorkshire2	704	90.7	0.2488	117	15.1	14.55
KWS Trinity	Yorkshire1	707	82.2	0.1370	126	16.9	14.05
KWS Trinity	Yorkshire2	727	86.9	0.2665	119	16.4	14.50
KWS Trinity	Lincolnshire	708	83.5	0.1906	127	14.2	14.63
KWS Trinity	Cambridge	715	84.7	0.1604	116	14.8	14.05
KWS Barrel	Lincolnshire	704	92.9	0.2337	122	14.9	14.26
KWS Barrel	Cambridge	709	87.5	0.1972	114	15.3	14.40
KWS Basset	Cambridge	726	87.4	0.1592	97	17.2	14.36
	SE	3.8	1.05	0.00958	2.4	0.38	SEM = 0.263

NSP = non-starch polysaccharides; Sol NSP = soluble proportion of total NSP.

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CONCLUSION

There was no difference in AME between six current UK feed wheat cultivars but growing site had an effect on nutrients composition of wheat cultivars.

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An investigation of the effects of provision of platform perches and dust-baths on welfare-related measures in commercial broiler chickens

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APPLICATION

1135 Platforms were consistently occupied across weeks, suggesting that provision enabled birds to fulfil an important behavioural need. The level of provision of platforms within this trial had no positive effects on broiler leg health.

INTRODUCTION

1140 Platforms are used to a greater degree by fast-growing broiler chickens than traditional perches (Bailie and O'Connell 2016). However, knowledge regarding the effects of the provision of platforms on the welfare of these birds is limited. The aim of the present study was to investigate the effects of providing platforms on the leg health and welfare of fast-growing commercial broilers. The provision of a suitable dust-bathing substrate also has the potential to increase activity levels, and to positively influence leg health in these birds (e.g. Reiter and Bessei 1998). Therefore, the effects of additional provision of peat-filled dust-baths were also assessed.

MATERIALS AND METHODS

1150 This study took place in N. Ireland between March and August 2016. Windowed houses containing ~23,000 as

hatched Ross 308 broiler chickens were assigned to one of 3 treatments: (1) control (C) (no platforms or dust baths), (2) platform perches (P) and (3) platform perches and dust baths (PD). In treatments 2 and 3, six platforms were provided per house, each measuring L230 × W90 × H20 cm. In treatment 3, 4 peat-filled dust-baths were provided per house, each measuring L230 × W90 cm, in addition to 6 Ps. Each treatment was applied in one of 3 houses on each of two farms, and was replicated over three 6-week production cycles. Treatments were balanced across houses during the three replications. Each week during weeks 3–5 of the rearing cycle, the severity of angular leg deformities (Letterier and Nys 1992), and of hock burn and podo dermatitis lesions, and walking ability (Welfare Quality® 2009) were recorded for 24 randomly selected birds per house, and video observations of perches (2 × 30mins per week) were taken. The incidence of dermatitis was also assessed at slaughter. The means of normally distributed data were compared using ANOVA in SPSS (version 22) with “cycle” and “house” as blocking factors and “enrichment” or “enrichment × week” as treatment factors. Average values per treatment, week (if applicable) and replicate were used as experimental units, and all main and interactive effects were determined along with the root mean squared error (RMSE), or the difference between models predicted and observed values. Tukey HSD post-hoc tests were carried out where appropriate. The study was approved by the School of Biological Sciences (Queen's University Belfast) Ethical Review Committee.

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Table. Main treatment effects on leg health parameters.

Parameter	C	P	PD	RMSE	P
% lame birds	9.27	13.66	12.27	14.03	0.64
Mean gait score	1.48	1.57	1.52	0.18	0.35
% birds with hock burn (at slaughter)	7.11	3.64	8.35	8.39	0.62
% birds with podo dermatitis (at slaughter)	24.51	27.12	25.19	3.32	0.94
Severity of angular leg deformities	0.70	0.74	0.69	0.23	0.75
Severity of hock burn lesions	1.21	1.24	1.26	0.04	0.62
Severity of podo dermatitis lesions	1.77 ^a	2.08 ^b	1.97 ^{ab}	0.35	0.04

RESULTS

The average numbers of birds occupying a platform perch was consistent across weeks (Wk3 26.64, Wk4 25.33, Wk5 26.26, RMSE 7.29, $P = 0.90$). There was no significant effect of the provision of platforms or platforms and dust-baths on any of the leg health parameters measured, with the exception of the severity of podo dermatitis lesions (Table). Severity was significantly increased in P birds compared with C birds ($P = 0.03$). The mean severity of podo dermatitis lesions in PD birds was intermediate and did not differ significantly from either those of C ($P = 0.23$) or P ($P = 0.61$) birds. There were no significant interactions found between enrichment and week.

CONCLUSION

Provision of platforms had no significant positive effects on broiler leg health. However, platforms were consistently occupied across weeks, suggesting that provision enabled birds to fulfil an important behavioural need. The reason for the rise in the severity of podo dermatitis lesions with the provision of platforms is not clear, but it was not reflected in the incidence of podo dermatitis and was partially mitigated by the provision of dust-baths. Further

research is required to determine the optimal level of provision of platforms required to positively influence welfare.

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Comparison of dustbathing and foraging enrichments for commercial broiler chickens

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IMPLICATIONS

Oat husks may be a suitable dustbathing enrichment for commercial broilers and promote more active behaviours than straw bales. The optimal level of provision required to improve overall welfare and the impact on airborne dust levels remains to be assessed

INTRODUCTION

Research suggests that broilers are capable of moving more when stimulated to, and that increasing activity in young birds can improve leg condition in adults (Bessei 2006). This study evaluated the effects of provision of environmental enrichment in the form of rings containing oat husks or/and straw bales on activity and welfare-related parameters in commercial broiler chickens.

MATERIALS AND METHODS

A total of 355,400 Ross broiler chickens (50:50 male and female; approx. 23,000 per house with 4 replicates) were randomly housed in one of four treatments for the duration of their 6-week cycle: (1) a control house (with no enrichment), (2) short-cut straw bales (SB) (2 per 1000 birds throughout the cycle; nine at any one time), (3) oat husks (OH) and (4) both straw bales and oat husks (OH +SB). Plastic wrapping on straw bales was cut open on day 10. Equivalent to the level of straw bale provision, nine steel rings containing oat husks were provided from day 10. Rings were 7.6 cm deep with a 1.1 m diameter containing approximately 9 kg of oat husks. All enrichments were replenished as necessary. Behavioural, leg health and environmental measures were recorded in weeks 3–6. Video footage of randomly chosen enriched and unenriched areas of the house was recorded weekly. In the enriched areas, scan sampling of the videos (10-min

intervals) was used to categorise the behaviour of birds directly engaged with the enrichment (inside the OH rings or in a predefined area around the SB). In unenriched areas, identical scan sampling categorised behaviour of birds within an equivalent predefined area on the screen. Behaviours were expressed as the % of the total birds scanned. Leg health was monitored using a modified gait scoring method (Garner *et al.* 2002), which was applied to two randomly selected birds from each of 20 sections of each house each week. Production records, including dermatitis levels, BW and mortality were obtained from company reports. To analyse the difference between use of OH and SB, overall values for birds directly interacting with the OH and SB were grouped from all birds interacting with each enrichment type in the OH, SB and OH + SB treatments. Behaviour in unenriched areas and all other parameters were analysed by treatment (OH vs. SB vs. OH + SB vs. C). Proportional use of oat husks vs. straw bales, environmental measures, gait scores and production measures were compared using ANOVAs with cycle as a random factor and the main and interaction effects of week (where appropriate) and treatment investigated. Comparisons of the mean behaviours (%) performed in unenriched areas of each treatment were made using Kruskal–Wallis tests. All methods were approved by the School of Biological Sciences Queens University Belfast Ethical Review Committee.

RESULTS

A comparison of behaviour when interacting with OH and SB showed that birds in the OH rings performed more active behaviours than those recorded around the SB (Table). Age effects were seen on birds in the OH rings, with an increase in % dustbathing ($P = 0.003$) and decrease in % foraging ($P < 0.001$) between weeks 3 and 6. Treatment had an effect on behaviour in unenriched areas of the house, with more locomotion ($P < 0.001$) and

Table. Comparison of bird behaviour when interacting with oat husks or straw bales. Mean percentage \pm SD.

Behaviours	Oat Husks	Bales	Pvalue
Dustbathing	14.04 \pm 11.18	0.33 \pm 1.71	0.019
Foraging	28.77 \pm 16.06	6.35 \pm 4.75	0.009
Sitting inactive	17.22 \pm 8.89	49.46 \pm 16.69	0.031
Sitting pecking	24.25 \pm 8.88	9.55 \pm 5.51	0.019

less sitting inactive ($P < 0.001$) and less sitting pecking ($P < 0.001$) performed in the C compared to enriched houses. % foraging was unaffected ($P > 0.05$). Treatment had no effect on any production or environmental measures ($P > 0.05$).

CONCLUSION

Oat husks appeared more successful in promoting dustbathing and foraging behaviours than straw bales, although straw bales are likely to provide beneficial cover for resting. However, no associated improvements in leg health or other production-related parameters were shown, even when both types of enrichment were provided. This may reflect a corresponding reduction in activity in unenriched areas of the house, and future research should focus on understanding correct level of provision of enrichment substrates such that overall beneficial effects are shown.

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Essential oils (crina and nutmeg) influenced intestinal morphology and microbial population of broiler chickens

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APPLICATION

Harnessing the EO of crina and nutmeg powder improve gut health of broiler chickens suggesting positive synergy among various active ingredients. Its inclusion in broiler diets is therefore advocated.

INTRODUCTION

Modification of gut microbiota population by eliminating harmful pathogens through competitive exclusion and enhanced proliferation of beneficial microbes

often results in improved intestinal morphology thereby enhancing nutrient absorption and utilisation for optimum growth performance and health status of poultry birds. Crina contains putative active ingredients including thymol, eugenol and piperine while nutmeg (*Myristica fragrans* H) contains putative active ingredients such as α - and β -pinene, sabinene, myristicin, elemicin, eugenol and safrole, whose activity have been investigated in other plant extracts (Brenes and Roura 2010). Due to the paucity of available information, this study aimed to evaluate the effect of crina and nutmeg, both singly and in combination as feed additives, on intestinal morphology and microbial population of broiler chickens.

Table. *Effect of essential oils on intestinal morphology and gut microbial count of broiler chickens (day 42)*

Parameters	Control	Nutmeg	Crina	Crina + nutmeg	SEM	P-value
Villus height (µm)	885.00 ^b	760.00 ^b	975.00 ^b	1225.00 ^a	98.35	0.0009
Crypt depth (µm)	230.00 ^a	185.00 ^b	199.17 ^b	205.00 ^b	9.39	0.0014
Villus crypt ratio	3.85	4.21	4.94	5.98	0.28	0.1400
Total bacteria count	5.40 ^a	5.10 ^a	3.60 ^b	3.00 ^b	0.58	<0.0001
<i>Staphylococcus aureus</i>	3.90 ^a	3.25 ^{ab}	2.75 ^b	2.80 ^b	0.27	0.0517
<i>Lactobacillus</i>	1.05 ^b	1.80 ^a	1.60 ^a	1.95 ^a	0.20	0.0002
<i>Salmonella</i> spp.	0.95 ^a	0.05 ^b	0.10 ^b	0.16 ^b	0.21	<0.0001

Superscript alphabets indicate significant difference ($P < 0.05$).

MATERIAL AND METHODS

240 1-day-old, mixed sex, Marshall broiler chicks were randomly allocated to one of 4 dietary treatments. Diets were a nutritionally balanced, corn-soybean mash supplemented with no additive (Control), or 100 g/t of crina, nutmeg, or crina + nutmeg. (Crina used is a commercial product by DSM Nutritional Products (UK) Ltd, while nutmeg seeds were purchased at a local market in Abeokuta, sun-dried and milled into a powdery form.) At day 42, 6 broilers per treatment were slaughtered and gastrointestinal tracts carefully excised. Histological gut samples from the jejunum were fixed in Bouin's solution, and after histological processing, stained with hematoxylin and eosin. Six readings each of villus height (VH) and crypt depth (CD) were taken per bird. Digesta was collected from the small intestine, placed in sterile sample containers and distilled water (30 ml) added individually to the samples. The samples were homogenised and enumerated for bacteria population on appropriate agar. Microbial counts were log transformed prior to statistical analysis. Data generated were analysed using ANOVA technique in a Completely Randomised Design (SAS for Windows, 9.1.3 portable version, Cary, NC, USA). Differences between means were determined by Tukey's HSD test at $P < 0.05$ level of significance.

broiler chickens (Table). Birds that fed crina + nutmeg had longer ($P < 0.05$) villi and higher villus crypt ratio than control diet. This indicates better gut health, improved digestion capacity and higher absorptive efficiency resulting in improved growth performance in such birds (Brenes and Roura 2010). Reduced CD with EO supplementation depicts lower epithelial turnover to maintain VH. In this study, improved gut balance by EO addition coincided with a significant improvement in bodyweight gain and reduced FCR in broilers (data not shown). Lower ($P < 0.05$) total bacteria count and higher *Lactobacillus* counts in birds that fed crina and crina + nutmeg suggests that they possess antimicrobial properties which could improve the survival and persistence of health-promoting microbes in the gut. Increased *Lactobacillus* could probably activate the intestinal immune system, increase resistance to diseases and improve the metabolism through increased digestive enzyme activity.

CONCLUSION

Birds that fed crina + nutmeg had longer villi, reduced CD and higher VCR than control diet birds. Reduced bacteria count and higher *Lactobacillus* counts were observed in birds fed crina and crina + nutmeg.

RESULTS

Essential oils (EO) addition significantly ($P < 0.05$) influenced intestinal morphology and microbial counts of

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The inclusion of whole grain wheat in the diets of growing turkeys

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APPLICATION

Adding whole grain wheat to the turkey diet reduces feed costs, which may outweigh losses in performance efficiency. It also reduces gizzard digesta pH, which may be beneficial for gut health.

(2003) and Plavnik *et al.* (2002) reported positive effects on bird performance, whereas Svihus *et al.* (2010) and Zduncyk *et al.* (2013) reported limited responses. The objective of this study was to determine the effects on bird performance and digesta pH when turkey poult were fed fixed proportions of wheat (0, 100 and 200 g/kg).

INTRODUCTION

Feeding whole grains to commercial poultry reduces feed costs and can improve bird gut health (Forbes and Covasa 1995). However, responses can be variable; Erener *et al.*

MATERIALS AND METHODS

Six-week-old turkeys (72) were blocked by liveweight and randomly allocated to one of three dietary treatments (4 pens per treatment, 6 birds per pen). The diets were a commercial pelleted ration (CON) and the same diet

Table. *Effect of whole wheat inclusion on the performance of Turkey poult*

	CON	LWGW	HWGW	SEM	P-value
<i>Phase 1</i>					
Feed intake (g/d)	360	346	365	9.0	0.352
Growth rate (g/d)	139 ^a	124 ^b	131 ^{ab}	3.6	0.013
FCR	2.635 ^a	2.916 ^b	3.036 ^b	0.042	0.001
<i>Phase 2</i>					
Feed intake (g/d)	473	468	473	13.8	0.705
Growth rate (g/d)	200	183	199	6.6	0.113
FCR	2.367 ^a	2.574 ^b	2.456 ^b	0.026	0.004

Table. *Effect of whole wheat inclusion on the digesta pH of Turkey poult*

Digesta	Phase						P-value		
	1			2			SEM	Phase	Diet
Site	CON	LWGW	HWGW	CON	LWGW	HWGW			
Gizzard	3.45 ^a	2.60 ^b	3.05 ^{ab}	3.34 ^a	3.38 ^a	3.06 ^{ab}	0.156	0.077	0.026
Caecum	5.78	5.78	5.49	5.89	5.74	5.49	0.179	0.858	0.140

thoroughly mixed with either 100 g/kg (LWGW) or 200 g/kg (HWGW) whole grain wheat (WG). Feed was offered **AL**. After three weeks (Phase 1), three birds from each pen were euthanised and samples of gizzard and caecal digesta were collected for determination of digesta pH. Three weeks later (Phase 2), all remaining birds were euthanised and similarly analysed. Bird liveweight and feed intake were recorded weekly. The effect of dietary treatment on bird performance and digesta pH was determined by **ANOVA** using a general linear model (Minitab 17, Minitab Inc), accepting $P < 0.05$ as significant.

RESULTS

Inclusion of WG in the diet did not affect feed intake, but did increase **FCR**. LWGW was associated with lower growth in Phase 1 (Table 1). WG reduced gizzard pH but had no significant effect on caecal pH (Table 2).

CONCLUSION

Feeding WG to young poult in this study negatively affected both bird growth and feed efficiency, whereas

feeding WG later in life seemed only to affect feed efficiency. Feeding WG reduced gizzard pH which has been associated with improved gut health and development.

ACKNOWLEDGEMENT

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Quantifying the effects of cuttlebones and an abrasive beak blunting object on beak shape and pecking force in two breeds of laying hens

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APPLICATION

Reducing beak length could affect the severity of injurious pecking damage, improve feather cover and reduce mortality rates for commercial hens. Abrasive materials for beak blunting may be an effective alternative to beak treatment.

INTRODUCTION

Reducing injurious pecking and the associated damage in laying hens is imperative to improve welfare. Though it does not solve the underlying causes, one approach to reducing feather damage and cannibalism is to provide

an abrasive surface to reduce beak length (Van de Weerd *et al.* 2006) or injurious pecking (Moroki and Tanaka 2016).

means \pm SE from LMM (back transformed means): 29 weeks 8.85 ± 0.06 (6967 milliNewtons), 35 weeks 8.55 ± 0.06 (5146 mN), 40 weeks 8.62 ± 0.06 (5552 mN).

MATERIALS AND METHODS

Thirty-six White Leghorn (WL) hens and 36 Columbian Rock (CR) hens with intact beaks were housed individually in barren cages from 29–40 weeks of age. Twelve hens of each breed were given one of two pecking objects (cuttlebone (Prevue Hendryx Pet Products, USA) or beak blunting board (S N Supplies, UK)), with half installed in the feed trough and the other half hung vertically on the side of the cage. The remaining 12 hens of each breed were housed without any pecking objects. Beak shape (photos taken and measured digitally using tspDig2 software (SUNY Stony Brook Morphometrics, USA) and mean peck force (measured via force plate (Bertec Corp., USA)) were collected at 29 weeks of age (prior to object installation), 35 and 40 weeks of age. Objects were replaced as needed. Data were analysed using Linear Mixed Models (with natural log transformation) (LMM) in Genstat (16th Edition) with $\alpha = 0.05$. All procedures were approved by SRUC's animal ethics committee.

RESULTS

Cuttlebones were replaced at least once for 12 of the 24 hens offered them (9 CR, 3 WL). There was no evidence to suggest the blunting boards were used. Beak length was affected by age ($P < 0.001$) and breed ($P = 0.011$, Fig.). Cuttlebones tended to reduce beak length ($P = 0.070$), but there was no effect of location ($P = 0.331$). CR hens had smaller beak tip angles (i.e. sharper tips) ($P < 0.001$; predicted means \pm SE from LMM (back transformed means): WL 4.25 ± 0.03 (69.76°), CR 4.04 ± 0.03 (56.88°)). Perpendicular peck force was affected by age only ($P < 0.001$), as it was the strongest at 29 weeks (predicted

CONCLUSION

Cuttlebones, but not the blunting boards, were attractive to some hens and showed encouraging results for shortening upper mandibles, though larger sample sizes may be required in future studies to obtain statistical significance as some hens that did not use the cuttlebones. Further studies are needed to assess the efficacy of cuttlebone access on injurious pecking behaviour and feather cover, as it is uncertain how beak shape affects this. However, the use of cuttlebones in particular may not be commercially practical as they are brittle and easily worn if used by the hens.

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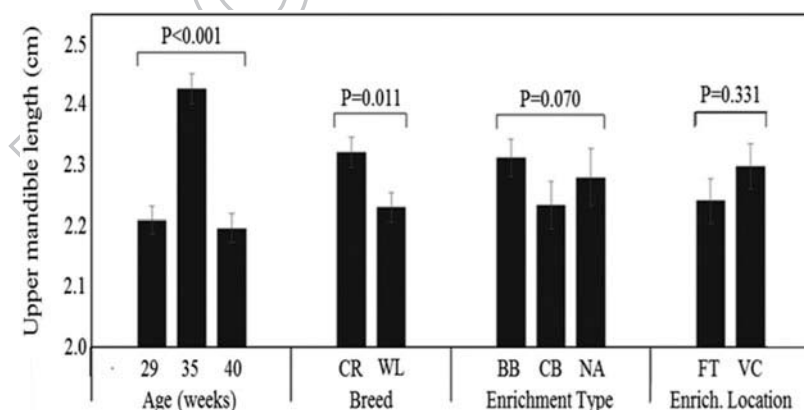


Figure 1 Mean upper mandible lengths (cm) predicted from LMM back transformed (\pm SE). Treatment factors: Columbian Rock (CR), White Leghorn (WL), Blunting board (BB), Cuttlebone (CB), No enrichment present (NA), Feed trough (FT), Vertical in cage (VC).

1535 Understanding the formation of the cuticle on eggs: a tool to improve egg safety?

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APPLICATION

1545 Understanding the physiology behind the formation of egg cuticle will allow us to develop strategies to be implemented to improve cuticle coverage and correspondingly improve the eggs natural antimicrobial barriers. The response of cuticle coverage to stress suggests it might make a sensitive indicator for monitoring flock health and welfare.

1550 INTRODUCTION

1555 Eggs have a layer of glycosylated protein on the outside of the shell known as the cuticle. It forms a defence to both vertical and horizontal transmission of micro-organisms that may threaten the viability of the developing embryo or cause spoilage of the egg. Previous studies (Bain *et al.* 2013) have demonstrated a positive correlation between cuticle quality and reduction in bacterial penetration of the egg. About 30% of cuticle variation is known to be genetic but little is known about other sources of variation. 1560 Confusion also exists regarding the relationship, if any, between the cuticle and pigment.

MATERIALS AND METHODS

1565 Lohmann brown egg laying hens were raised in pens (12 birds per pen) or transferred to individual cages on a set lighting programme (14L: 10D) prior to experimentation. All studies were carried out under project licence 7007909 with the Roslin institute ethics committee approval for each individual experiment. Hens were put on a 14L:14D ahemeral lighting pattern to synchronise ovulation and oviposition which allows the accurate determination of oviposition time. Cuticle deposition and pigment (colour) were quantified using reflectance spectroscopy of the unstained and eggs stained with Tartrazine/Lissamine green. A number of experiments 1575 were performed to determine critical events in the deposition of cuticle on the eggshell: (1) pen to cage transfer ($N = 24$); (2) comparison of premature oviposition using gonadotrophin-releasing hormone (GnRH) allowing an accelerated, but normal endocrine cascade leading to oviposition or arginine vasotocin (AVT) invoking only the last stages of the endocrine cascade leading to oviposition ($N = 12$, with each bird receiving all treatments thus acting as its own control); (3) time of AVT injection prior to expected oviposition ($n = 12$, each bird receiving all treatments); (4) the effect of the pigment removing coccidiostat nicarbazin ($N = 12$, each bird receiving both treatments). ANOVA (Genstat 13) was used for statistical analysis. least significant difference was used to test difference between means where appropriate. $P < 0.05$ was considered significant. 1590

RESULTS

Pen to cage transfer was accompanied by a small but significant ($P = 0.049$) decline in cuticle coverage but not in pigment deposition. Eggs prematurely induced with AVT to oviposit at the same time as a premature GnRH-induced 1595 oviposition had virtually no cuticle ($P < 0.001$), whereas GnRH-induced eggs had a normal cuticle. A similar pattern was observed in cuticle deposition. Eggs that prematurely oviposited at 1, 3 and 5 h prior to the expected oviposition time had little or reduced cuticle. Pigment was almost fully deposited in eggs prematurely oviposited 1 h compared to 3 and 5 h prior to the expected time of oviposition ($P < 0.001$). Nicarbazin resulted in the disappearance of pigmentation over a 7 day period ($P = 0.001$), while cuticle cover displayed a small but significant ($P = 0.004$) increase 1600 in coverage over the same period. 1605

CONCLUSION

Mild stress caused by pen to cage transfer has limited but significant effects on the cuticle which might be exploited to monitor flock health and welfare. The contrast between 1610 eggs oviposited prematurely by GnRH and AVT suggests that a normal endocrine cascade, as produced by GnRH, is a requirement to produce a fully formed cuticle. The premature oviposition induced by AVT, which essentially occur in the shell gland, is not sufficient to stimulate deposition of cuticle. It appears as if the cuticle is deposited in the very last moments before oviposition and the event is not positively correlated with pigment deposition, so confirming a lack of correlation between pigment and cuticle quantity. Overall, the study suggests that disturbance can reduce cuticle quality and reduce the effectiveness of the antimicrobial defence of eggs and this may add to the non-genetic variance observed in cuticle quality. 1615 1620

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1635 A new approach to knowledge exchange and innovation in practice

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APPLICATION

1640 Establishing practice-driven innovation networks could benefit agricultural practice, profitability and sustainability based on increased adoption of applied science.

INTRODUCTION

1645 There is growing evidence that, despite considerable investment in knowledge transfer, there remains a gap between scientific research and the embedding of applied science into farm practice (e.g. Hill *et al.* 2017). The Hennovation project has been testing mechanisms to both enhance the uptake of scientific knowledge and enable practice-driven innovation. The new approach 1650 establishes innovation networks of farmers or those engaged in the laying hen processing industry that are facilitated to proactively search for, share and use new ideas to improve hen welfare, efficiency and sustainability. Such collaborative learning approaches are proposed to 1655 have greater potential for uptake than “top-down” approaches (MacMillan & Benton 2014) and this is also being evaluated.

MATERIALS AND METHODS

1660 Supporting networks requires skilled facilitation. Thus, 11 facilitators in 5 EU countries (UK, Sweden, Netherlands, Spain and Czech Republic) had initial training to understand the innovation process and to both mobilise and support grassroots networks. A framework for the adaptive 1665 management and facilitation of practice-driven innovation was developed through participatory research and collective learning among network facilitators. Social scientists evaluated the whole process of the new methodology and how innovative ideas were generated, tested and refined in practice within the networks by analysing the online Wiki 1670 that recorded network progress, attending workshops and network meetings as well as holding structured interviews with each facilitator.

RESULTS

1675 Some 15 innovation networks, involving producers and laying-hen processors, have been mobilised in the 5 countries and are involved in finding solutions to concerns such as controlling red mite, minimising injurious pecking, marketing hen meat and improving handling practices at the end of lay. Responding to cultural norms and recognising that each network has unique needs for support has 1680 been an important outcome in the learning process. The networks are all multi-actor and have been assisted by a variety of specialists including animal welfare scientists, veterinary surgeons, technical experts and food chain

1685 actors. Moreover, most have received small grants to support their trials.

The key steps necessary for local innovation have been established and include identifying a problem, generating an innovative idea, agreeing on and focussing on one idea at a time, planning and resource mobilisation, 1690 trialling the innovation, implementing/upscaling and finally dissemination/embedding. In addition to these process steps, the project has identified some important conditions necessary for supporting innovation within practice-led networks. These include involving the right 1695 people in the network, identifying common goals, focusing on areas that can change, providing sufficient resources, learning by doing, using knowledge from within and outside the network and crucially, expert facilitation.

CONCLUSION

1700 Expert facilitation and sufficient resource is needed to support the establishment of practice-led networks in order to realise their potential for embedding science, developing innovative solutions and sharing best practice 1705 to drive productivity in the sector.

ACKNOWLEDGEMENTS

The paper draws upon research and discussions conducted under the HENNOVATION project, a H2020 EU collaborative research project with 6 academic partners funded under the topic “Innovative, Sustainable and 1710 Inclusive Bioeconomy” ISIB-2-2014/2015: Closing the research and innovation divide: the crucial role of innovation support services and knowledge exchange, grant agreement no. 652638. The authors wish to thank the many people involved in that project who collaborated in 1715 that research and contributed to the material of this paper.

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The use of insects in the animal production sector with an insight into consumer perception of insects as food or feed and potential to replace antibiotics

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INTRODUCTION

It is widely accepted that by 2050 the world will host 9 billion people. To accommodate this number, current food production will need to almost double. The livestock feed market is large and growing; global demand for animal feed is estimated to be worth £236 billion. There is a growing interest in alternatives to traditional livestock feed – soya bean, fish meal and other processed animal protein (PAP) – which bring significant environmental and, as a result, financial costs. Projections of global meat demand suggest massive increases so there is a concern to find new ways to address this. Alternatives have to have high protein content with the right amino acids and be digestible and palatable to the livestock.

The UN's Food and Agriculture Organisation has identified that insects could have a valuable role to play in this both as a component of human diets and as a source of feed for livestock. As result, there is a growing body of research into the many different aspects of this – usefully reviewed recently by Dossey *et al.* (2016). There is a wide spectrum of research in the UK and Europe looking into sustainable protein, including calls through the Sustainable Agriculture and Food Innovation Platform, Innovate UK and Horizon 2020.

Studies have established that insects will not be able to match the nutritional characteristics of fish meal; however, insects could become a major animal feed source. House fly and black soldier fly are rich in protein and have clear potential as a protein source in animal nutrition. Additional nutritional components that add value to insect products include fats/oils and vitamins & minerals. As a result, insect meals could partially replace fish meal for some livestock and may even be able to completely replace some vegetable or soy meals for monogastric livestock (pigs, poultry). The PROteINSECT programme conducted poultry feeding trials and the results indicated that no significant differences could be observed in animal performance. Insects are also highly efficient in the rapid conversion of a range of "waste" substrates into biomass and they require much less land than equivalent quantities of feed alternatives. Insect-based feed products could have a similar market to fishmeal and soy, which are presently the major components used in feed formulae for aquaculture and livestock.

The use of insects as feed is a relatively new practice on a commercial scale, and many questions remain to be tackled, particularly regarding safety concerns. However, the European Food Safety Authority scientific assessment of the possible use of insects in feed believe the evidence suggests that when currently allowed feed materials are used to feed insects, the possible occurrence of any

microbiological hazards should not pose any additional risk compared to other feeds. In the European Union, the use of insects as a source of protein for animal feed for animals raised for human consumption is currently not possible due to requirements under Regulation EC 999/2001. Under EC regulation 1069/2009, insects reared for the production of PAP would currently be considered "farmed animals" and are therefore prohibited from being fed on manure or catering waste. The Commission has indicated, however, that they will allow insect protein to be fed to fish by summer 2017. We expect this to be extended to other livestock shortly. The cost of farming insects is the second major factor making the insect protein a high cost alternative to traditional feeds and research is underway to develop technologies to make insect farming less labour-intensive and more cost-effective.

PROteINSECT carried out consumer perception research and found a high level of support for insects as a protein source in animal feed. They also found many who would like more information on this topic. On behalf of the UK Government Office for Science, which also found similar views in the context of a discussion about options for addressing the major challenges facing food production – issues were raised about what the insects might be fed on.

One recent development that could significantly increase the value to the farmer (and consumer) of insects as feed is a technology, Immunity Generation, that can stimulate insects to create the antimicrobial peptides (AMPs) which enable them and creatures that eat them to resist diseases. This has been proven at scale by studies carried out by the School of Veterinary Science, University of Bristol. The studies focused on establishing resistance in poultry to *Campylobacter* and members of the family of bacteria that include *Salmonella* and *E. coli*. When stimulated, insects are fed to livestock, the AMPs are accepted by the poultry in their guts and confer immunity to a range of poultry-associated diseases. This approach can be varied to stimulate the production of different AMPs, with the expectation that this would mean they could be targeted at different livestock and different pathogens. It could be tested to see if it would work with other animals. Trials are also needed to see if it could be used to protect livestock from viruses such as avian flu.

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Rapeseed cultivar and micronising affect the metabolisable energy of rapeseed meal for broilers

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APPLICATION

Micronising only improved the energy value of low ME rape seed meal.

was also recorded. A randomised complete block ANOVA was performed and a 2 × 4 factorial structure was used to investigate the main treatment factors (the presence of micronizing and four RSM cultivars) and their interaction. Differences were reported as significant at $P < 0.05$.

INTRODUCTION

Rapeseed meal (RSM) is a widely available and valuable protein source that may offer a sustainable alternative to SBM. The AME of RSM is lower than that of SBM, which currently limits its inclusion in broiler feed (Khajali and Slominski 2012). Commercial RSM is produced from a mixture of rapeseed cultivars and these may vary in their ME content. This study investigated differences in the AME of broiler diets, formulated with RSM produced from different UK-grown cultivars. The effect of micronising on energy utilisation was also explored.

RESULTS

Overall, the AME of diets formulated with RSM produced from cultivar Picto was significantly lower than the AME of the remainder of the diets ($P < 0.05$). A significant interaction was observed between cultivar and micronising ($P = 0.05$), and in cultivar Picto, AME was significantly improved following micronizing ($P < 0.05$). The AME of the remaining diets remained unchanged. The observed responses were likely caused by variation between cultivars in the chemical characteristics of seeds. Lawrence (1978) reports that micronising led to the deactivation of glucosinolates and improved the available energy of RSM for pigs. With broilers micronizing increased the ME in canola-quality *Sinapis Alba* mustard seed (Slominski *et al.* 1999) possibly due to better utilisation of the NSP fraction (de Vries *et al.* 2012).

MATERIALS AND METHODS

The experiment was approved by the Animal Experimental Committee of Harper Adams University. Four cultivars of UK-grown whole rapeseed (Hawai, Compass, Picto and Popular) were obtained. Seeds were cold-pressed (KK20; Kern Kraft GmbH, Germany), and the milled presscake was subjected to 10 h of continuous Soxhlet extraction with hexane. The marc was then transferred to fume hoods for air desolventisation. Defatted material from each cultivar was divided and half underwent heat treatment by micronising (carbon infrared emitters at 125°C for 60 s; Heraeus Noblelight GmbH, Germany). For diet preparation, a standard broiler starter feed was replaced by 25% of the respective RSM products (w/w), standardised to contain 3% fat. Day-old male Ross 308 broiler chicks were obtained from a local hatchery and housed in an environment-controlled room. Lighting and temperature settings conformed to breeder specifications (Aviagen, Edinburgh, UK). All birds received a standard mash broiler starter diet from day 0 to 13 and access to feed and water was provided *ad libitum*. On day 13, all birds were assigned to one of 36 raised floor pens (5 birds per pen). Each diet was fed to 6 pens following randomisation. Excreta were collected quantitatively for the last four days of the study from 17 to 21 d age and feed intake

CONCLUSION

Micronising only improves the energy value of RSM for broilers, when produced from cultivars with a relatively low ME content. Improving transparency and traceability in the RSM production chain would enable feed producers to select the meals with the highest AME value for poultry.

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Table. The effect of micronizing and rape seed cultivar on dietary apparent metabolisable energy (AME MJ/kg DM) when fed to broilers.

Micronizing	Cultivar				SEM	P-value
	Charger	Hawai	Picto	Popular		
No	13.53	13.74	13.05	13.55	0.091	0.006
Yes	13.52	13.53	13.49	13.36		
Cultivar means	13.53	13.63	13.27	13.46	0.065	0.005

The inclusion of processed soya in the starter diet for broilers improves 35-day performance

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APPLICATION

This data will help inform formulators and nutritionists on the most beneficial and economical use of extruded soya products for broilers.

INTRODUCTION

In recent years, there has been increasing interest in formulating higher-quality starter diets for broilers and in some systems the use of short-term pre-starter diets (Barekattain and Swick 2015). The objective was to increase early BW which was correlated with end bodyweight and to improve gut development, by providing higher-quality ingredients. Anti-nutritional factors such as trypsin inhibitors can limit the inclusion of soya products. Secondary processing, such as extrusion, can further reduce such ANFs allowing improved performance from soya (Clarke and Wiseman 2007). This experiment was designed to test the inclusion of a novel extruded soya product in either starter diets, or for the entire broiler cycle, on growth performance of female Ross 308 broilers. The novel soya product, AlphaSoy 530 (AgroKorn, Denmark), is produced using an extrusion-based process. Such processed soya ingredients are common for piglets and less so for broilers.

MATERIAL AND METHODS

Two hundred and four, 1-d-old, female Ross 308 broilers were assigned to 34 cages, 6 birds per cage. Three diet phases were fed: 0–10, 11–25 and 26–35. Two diets were manufactured for each stage, either containing AS530 at 20% or HiPro soya as a source of protein. Diets were corn/wheat/soya-based and formulated to be as isocaloric and isonitrogenous as possible. Birds were allocated to one of three treatments, soya diet throughout (control, A), novel soya in the starter only (B) or novel soya throughout (C). For example, the basal starter diet contained corn (32%), wheat (30%), HiPro soya (32%), soyabean oil (2.4%) and the remainder was vitamin and mineral products. Growth performance was measured at days 10, 25 and 35. Water intake was measured between days 15–17 and 29–31 and faecal quality was measured at day 22. Treatment A was fed to 12 replicate cages and B and C to 11 each. This study was given ethical approval by the trial site ethical committee.

RESULTS

Final bodyweight of the control birds were ahead of the breeder guidelines (2014; 2006 g at 35 d). There were no

significant differences in faecal score or water intake ($P > 0.1$). Growth performance data has been selected for brevity (Table). Where no data is shown (e.g. feed intake (FI)), there were no significant differences. The inclusion of a novel processed soya in the starter diet only, compared with a soya-only diet or providing the novel product throughout the cycle, significantly improved daily BWG between days 25 and 35. It is possible that having the novel product in the starter diet helped to prepare birds for HiPro soya in the following diets. It is unclear why those birds that fed the novel product throughout were not significantly improved to the same extent. It is possible that there was an effect of the novel product when fed throughout (C), but it was not detectable because the addition is confounded by the removal of fat (in this case approximately 1% absolute). This removal of fat may have caused a concurrent decrease in performance so that the overall effect is equivalent performance to the control. AS530 has an improved ME value relative to soya (Navarro 2014). However, this also indicates an alternative, lower fat formulation, which is possible with the use of AS530, with no significant loss of performance.

CONCLUSION

Extruded soya, such as AS530, can be successfully formulated into broiler diets at up to 20%, as a partial replacement for HiPro soya. The most cost-effective use may be in the initial diet, to day 10, which may result in improved BWG at the end of the cycle.

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Table. Growth performance in body weight gain (BWG), body weight (BW) and feed conversion ratio (FCR).

	A	B	C
BWG 0–10 (g/d)	26.3	25.4	25.9
BWG 25–35 (g/d)	76.3b	82.4a	76.6b
BWG 10–35 (g/d)	70.2B	72.6A	69.5B
BW d35 (g)	2065	2116	2044
FCR 0–35	1.522	1.525	1.532

^{a,b} $P < 0.05$, ^{A,B} $P < 0.10$.

2000 Effect of L-Dopa on performance and lipid profile of broiler starter

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APPLICATION

2005 L-3,4-dihydroxy-phenylalanine (L-Dopa) extract at 1-4 g/kg inclusion levels had no detrimental effect on growth response of birds, however, 2 g/kg is recommended.

INTRODUCTION

2010 Mucuna pruriens is a tropical legume with nutritional quality comparable to soybeans, but its utilisation as live-stock feed has been limited due to presence of anti-nutritional factors like tannins, phytic acid and L-3,4-dihydroxy-phenylalanine (L-Dopa). L-Dopa is the principal precursor for dopamine and catecholamines. Dopamine stimulates the hypothalamus and pituitary to release and increase the level of growth hormone production while catecholamines are powerful stimulators of triglycerides from adipose tissue and exert their effect by binding to adrenergic receptors and stimulating fat oxidation. Feeding raw mucuna seeds to broilers resulted in reduced nutrient absorption, structural disruption and collapse of intestinal microvilli (Iyayi *et al.* 2008). However, L-Dopa-rich extracts from *Mucuna pruriens* was considered to improve the performance and carcass quality of birds (Vadivel and Pugalenthil 2010; Omidiwura *et al.* 2016). The aim of the study was to determine the effect of L-Dopa on the performance and lipid profile in broiler chickens.

MATERIALS AND METHODS

2030 Two hundred and forty day-old Abor Acre broiler chicks were weighed and randomly allotted to 6 diets. The diets contained standard energy (SE) + 0 g/kg L-Dopa, high energy (HE) + 0 g/kg L-Dopa, HE + 1 g/kg L-Dopa, HE + 2 g/kg L-Dopa, HE + 3 g/kg L-Dopa and HE + 4 g/kg L-Dopa. Each diet had 4 replicates of 10 birds in a complete randomised design. The starter diets were offered to the birds from day old to day 21. FI, BWG and FCR were assessed. On day 21, blood samples (2.5 mL) were collected from 2 birds per replicate from the jugular vein into set of bottles without EDTA to obtain serum for total cholesterol, high density lipoprotein (HDL), low-density lipoprotein (LDL) and triglyceride (TAG) analyses.

Data obtained were analysed with ANOVA (SAS 2012); means were separated using Duncan's Multiple Range Test ($P = 0.05$).

RESULTS

2045 The effect of L-Dopa supplementation on the performance and serum lipid profile of birds is shown in the Table. BWG of birds fed SE + 0.0 g/kg L-Dopa diet were similar to those of birds on HE + 1 g/kg L-Dopa but significantly higher than birds on other diets. FI of birds that fed SE + 0 g/kg L-Dopa diet were higher ($P < 0.05$) 2050 than FI of birds on other diets with the least recorded in birds fed HE+4 g/kg L-Dopa. The FCR of birds were not affected by the diets. The serum TC of birds that fed SE +0 g/kg L-Dopa, HE+0.0% L-Dopa and HE + 4 g/kg L-Dopa diets were similar to that of birds that fed HE + 0.1% L-Dopa diet but higher than those birds that fed the other diets. The LDL of birds that fed SE+0 g/kg L-Dopa and HE+0 g/kg L-Dopa were significantly different but similar to those of birds on other dietary treatments. Similar trend was observed in the serum triglyceride of birds on experimental diets. However, dietary treatment did not significantly affect HDL. 2060

CONCLUSION

2065 Supplementation of L-Dopa in high energy broiler starter diets at 1-4 g/kg inclusion levels did not elicit any deleterious effect on the performance of birds. However, L-Dopa inclusion at 2 g/kg is recommended for broilers at starter phase.

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Table. Performance (g/bird) and lipid profile (mg/dL) of starter broilers on experimental L-Dopa diets.

Period	Diets (g/kg)	FI	BWG	FCR	TC	LDL	HDL	TAG
Starter 0-21d	SE+0.0 L-Dopa	978 ^a	680 ^a	1.44	125 ^a	78 ^b	115	78 ^b
	HE+0.0 L-Dopa	773 ^{cd}	539 ^c	1.44	129 ^a	134 ^a	105	134 ^a
	HE+1.0 L-Dopa	858 ^b	624 ^{ab}	1.38	121 ^{ab}	99 ^{ab}	118	99 ^{ab}
	HE+2.0 L-Dopa	839 ^{bc}	605 ^b	1.39	117 ^b	110 ^{ab}	98	110 ^{ab}
	HE+3.0 L-Dopa	711 ^{cd}	521 ^c	1.37	116 ^b	112 ^{ab}	112	112 ^{ab}
	HE+4.0 L-Dopa	681 ^c	478 ^c	1.43	131 ^a	113 ^{ab}	116	113 ^{ab}
	SEM	22	21	0.03	6	13	10	13
	Pvalue	0.001	0.001	0.309	0.0063	0.0296	0.3955	0.0296

Means on the same column with different superscript alphabets are significantly different ($P < 0.05$). SEM = standard error of mean.

Inter-relationship of microbial phytase and myo-inositol on growth performance, energy partitioning of male and female broiler chickens when fed a wheat-soy-based diet designed to have a relatively low available phosphorus (P) content

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APPLICATION

Exogenous phytase (PHY) and myo-inositol (MYO), alone or in a combination, may benefit broilers.

INTRODUCTION

When MYO, the final product of phytate dephosphorylation, is added to a P-deficient diet, it improves the growth performance of broilers (Żyła *et al.* 2004), which suggests that a part of the enhanced performance of PHY supplementation is due to release of MYO. Therefore, the objective of this study was to investigate the effects and inter-relationship of PHY and MYO when fed to male and female broilers.

MATERIALS AND METHODS

A total of 64 male and 64 female Ross 308 broiler chickens were used in this study. A wheat-based control diet (C) was formulated to be adequate in protein (240 g/kg CP) and energy (12.80 MJ/kg ME) but lower in non-phytate P content (3.3 g/kg diet). The basal diet was then split into two batches and one of them was supplemented with 500 units/kg (FTU) of an enhanced E coli 6-

PHY (Quantum Blue). The two batches (with and without PHY) were then split into four equal parts each and were supplemented with pure MYO (Sigma-Aldrich, USA) at 0, 1, 2 and 3 g/kg diet, respectively. Each diet was fed to 4 pens with males and 4 pens with females, with 2 birds each pen, to give a total of 16 experimental treatments in a randomised block design. The MYO and the PHY were added to the diets in powdered form and all diets had TiO₂ as indigestible marker and were fed as a mash. At day 21, all birds were killed by cervical dislocation and minced to determine the retained energy in the carcass. Data was analysed by ANOVA as a 4 × 2 × 2 factorial arrangement of treatments. The Animal Experimental Committee of Harper Adams University approved the study. Differences were reported as significant at $P < 0.05$ and trends were noted when P -value was near to 0.10.

RESULTS

Dietary supplementation with MYO had no effect on growth performance parameters ($P > 0.05$) but tended to reduce nitrogen-corrected AME (AMEn MJ/kg DM) ($P = 0.07$, linear) and it also had no effect ($P > 0.05$) on dietary digestible energy (DE) values. However, dietary supplementation with PHY resulted in a higher DMI

Table. The effect of dietary supplementary inositol and phytase on daily dry matter intake (DMI), weight gain (WG), feed conversion ratio (FCR), nitrogen corrected apparent metabolisable energy (AMEn), digestible energy (DE) and net energy (NE), in broilers.

Treatments	DMI (g/b/d)	WG (g/b/d)	FCR-DM	AMEn (MJ/kg DM)	DE (MJ/kg DM)	NE MJ/kg DM
MYO. 0	48.590	31.627	1.655	13.947	16.211	9.944
MYO. 1	48.015	32.301	1.592	13.860	15.694	10.288
MYO. 2	48.266	31.879	1.625	13.893	16.003	9.753
MYO. 3	48.667	32.281	1.617	13.717	15.698	10.086
SEM (df = 45)	1.1237	1.0255	0.0289	0.0802	0.2071	0.1551
PHY (-)	46.658	28.420	1.750	14.031	14.951	9.852
PHY (+)	50.110	35.625	1.494	13.678	16.852	10.184
SEM (df = 45)	0.7946	0.7251	0.0204	0.0567	0.1465	0.1096
Female	47.994	30.911	1.669	13.894	15.868	9.635
Male	48.775	33.134	1.575	13.814	15.935	10.401
SEM (df = 45)	0.7946	0.7251	0.0204	0.0567	0.1465	0.1096
<i>Probabilities of differences</i>						
MYO	0.975	0.959	0.505	0.228	0.233	0.113
Lin.	0.924	0.739	0.529	0.074	0.191	0.873
Quad.	0.666	0.895	0.354	0.580	0.611	0.97
PHY	0.004	<.001	<.001	<.001	<.001	0.039
SEX	0.491	0.035	0.002	0.322	0.75	<.001
MYO × PHY	0.951	0.704	0.333	0.318	0.16	0.201
MYO × SEX	0.508	0.707	0.116	0.045	0.701	0.019
PHY × SEX	0.433	0.628	0.862	0.263	0.109	0.571
MYO × PHY × SEX	0.988	0.956	0.843	0.299	0.899	0.632

($P < 0.01$), WG ($P < 0.001$) and a lower FCR-DM value ($P < 0.001$). Dietary supplementation with PHY resulted in a lower dietary AMEn value ($P < 0.001$) but surprisingly resulted in an increase in dietary DE value ($P < 0.001$). No sex effects were noted on DMI, but female chicks had a lower WG ($P < 0.05$) and a higher FCR-DM ($P < 0.01$) compared to male chicks. Dietary supplementation with MYO had no effect ($P > 0.05$) on dietary net energy values (NE MJ/kg DM), whereas supplementation with PHY resulted in a higher NE values compared to non-PHY diets for both male and female birds. Higher dietary MYO-fed males had higher NE values compared to females ($P < 0.01$).

CONCLUSION

Feeding phytase to broilers improved performance and energy availability of diets that were clearly severely deficient in P. Under such circumstances, MYO supplementation benefited males more than females which may relate to their greater growth potential and thus retardation due to the P deficiency.

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The use of near infrared reflectance spectroscopy (NIRS) to predict the nutritive value of wheat for broilers

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APPLICATION

Near-infrared reflectance spectroscopy can accurately predict performance of broilers when offered a particular wheat-based diet.

INTRODUCTION

Near-infrared reflectance spectroscopy (NIRS) has been shown to be effective in predicting the nutritive value of forage for ruminants and previous work by the Agri-Food and Biosciences Institute (AFBI) has indicated that it has the potential to accurately predict how a bird will perform when offered a diet based on a particular wheat (Owens *et al.* 2009). NIRS may therefore be a useful tool to manage the impact of batch to batch variation of wheat on broiler performance. The aim of this project was to validate existing NIRS equations (Owens *et al.* 2009), using birds accommodated in pens, to provide a tool to predict the nutritive value of wheat for broilers.

MATERIALS AND METHODS

This work was approved by the AFBI Animal Welfare Ethical Review Body. The results of three performance trials conducted at separate sites (AFBI, Harper Adams University (HAU) and Moy Park Ltd (MP)) were combined to create a database of FI, liveweight gain (LWG) and FCR values for broilers offered different wheat-

based diets. The AFBI trial evaluated 20 wheat samples, the MP trial evaluated 6 commercial feed wheat samples and the HAU trial evaluated 6 of the same wheat samples used in the AFBI trial. At AFBI, 1200 male broilers were in pens of 10 from 0 to 35 d, giving 6 pen replicates/wheat. For the Moy Park trial, 18,000 as-hatched broilers were in pens of 500 from 0 to 38 d, giving 6 pen replicates/wheat. At HAU, 960 male broilers were in pens of 20 from 0 to 42 d, giving 8 pen replicates/wheat. The 26 wheat samples evaluated were diverse in terms of location grown, variety and chemical composition. All diets contained at least 600 g/kg wheat and were formulated into starter, grower and finisher broiler diets to meet standard nutrient recommendations. The wheat samples used in all the trials were scanned as whole undried kernels on a Foss NIRSystems 6500 spectrophotometer. These scans ($N = 26$), along with the wheat scans from Owens *et al.* (2009) ($N = 84$), were analysed using Foss software. The mathematical treatment of standard normal variate and detrend, first derivative, gap of 4 and smooth of 4 was applied. Modified partial least squares regression was performed on the data set on the range 400–2500 nm and NIRS calibration and validation statistics generated to directly predict performance and compare with actual performance values obtained through the broiler trials.

RESULTS

The calibration and prediction of FI, LWG and feed efficiency (expressed as % gain:feed) were robust with

Table. The calibration and validation statistics for the prediction of broiler performance

	N	Mean	SD	Min	Max	SEC	R ²	SECV	SECV as % of mean	RPD cal*
FI (g/d)	182	78.7	10.1	48.2	108.8	2.77	0.92	3.08	3.9	4.12
LWG (g/d)	182	57.3	6.8	37.0	77.7	2.33	0.88	2.81	4.9	2.41
Gain:feed (%)	181	73.2	5.2	57.6	88.7	1.72	0.89	2.11	2.9	2.46

*RPD cal = ratio of prediction to deviation for calibration = SD/SECV (values above 3.0 indicate prediction equations are excellent).

Table. *Statistics of cross validation for feed intake, LWG and gain:feed (%)*

	FI (g/d)		LWG (g/d)		Gain:feed (%)	
	Actual	Predicted	Actual	Predicted	Actual	Predicted
Standard error of prediction (SEP)	2.96		2.79		2.02	
Means	78.7	78.5	57.3	57.1	73.2	73.1
R ²	0.92		0.83		0.85	
SEP as % of mean	3.8		4.7		2.8	
Standard deviation	10.15	9.70	6.73	6.49	5.23	4.86
RPD validation	3.43		2.41		2.59	

*RPD validation = ration of prediction to deviation = SD/SEP (values above 3.0 indicate prediction equations are excellent).

R² of 0.92, 0.88 and 0.89, respectively (Table 1). The SECV were low (3.08, 2.81 and 2.11, respectively) as were the SECV of the mean (3.9, 4.9 and 2.9, respectively) indicating that there is good correlation between actual and predicted values. In addition, RPD for FI indicated that the calibration prediction was excellent (4.12). The independent cross-validation statistics showing the strength of relationship between actual and predicted performance are presented in Table 2. The relationship between actual and predicted FI (R² = 0.92), LWG (R² = 0.83) and gain:feed (%) (R² = 0.85) were strong and SEP low (2.96, 2.79 and 2.02, respectively). The SEP as % of the mean were also low (<5% for all parameters) which indicates that the error in predicting the relationship is acceptable. The best RPD value for FI (3.43) and the RPD values for LWG and FCR indicated the predictions were quantitative and good, respectively.

The potential of microwave ashing for bone mineralisation measurement

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APPLICATION

Use of a traditional, electric element muffle furnace to determine bone ash content is time consuming with a high energy cost. Recent developments in microwave technology provide an alternative method of ashing bones for quantification of bone mineralisation, but do not offer appreciable time benefits.

INTRODUCTION

Assessment of bone ash content is frequently used in the poultry sector as a measure of the mineral portion of the bone to provide an indication of bone integrity. The standard methodology is to use a muffle furnace (MF) with electric heating elements set at 650°C for 13 h to ash the bones (AOAC 2000; Hall *et al.* 2003). Microwave ashing systems (MAS) are relatively new to the market and are advertised as an efficient alternative to convention furnaces, with both shorter running times and reduction in cooling times. Poultry bone ash has not been assessed using this process although a manufacturer estimated that bones would take an hour to ash. However, the MAS under consideration (one comparable in price to a small MF) only holds 8 tibia samples, so would need to be

CONCLUSION

Strong prediction equations were developed using data from birds kept under commercial conditions. These equations can accurately predict how birds will perform when offered a diet based on a particular wheat. The prediction equations could be further strengthened using additional samples and could then be made commercially available.

ACKNOWLEDGEMENT

DAERA, Moy Park Ltd. and ADHB Cereals and Oilseeds.

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considerably quicker than a MF in order to compensate for the loss of capacity. Therefore, the aim of this study was to directly compare the practical viability of a MAS with a conventional MF for bone ash quantification.

MATERIALS AND METHODS

Left and right tibias were collected from 12 male broiler birds aged 35 days. The birds were raised on a commercial broiler farm and fed a standard commercial diet. All bones were autoclaved and then de-fleshed by hand. Each bone was then dried for 24 h, defatted with petroleum ether, re-dried and weighed into a standard 50-mL ceramic crucible. The left tibia from each bird was ashed in a Genlab oven, model MINLO-50 conventional MF for 13 h at 650°C as per standard practice at NTU for bone ash determination. The right tibia from each bird was ashed in the PYRO XL MAS (Milestone Inc) according to the manufacturer's instructions. The microwave samples were re-weighed hourly up to 7 h. For both methods, ash percentage calculated as a proportion of dry bone weight. Each MF or MAS time point was considered a treatment factor, data were analysed via ANOVA with a Bonferroni *post hoc* test using IBM SPSS version 23, and statistical significance was declared at $P < 0.05$.

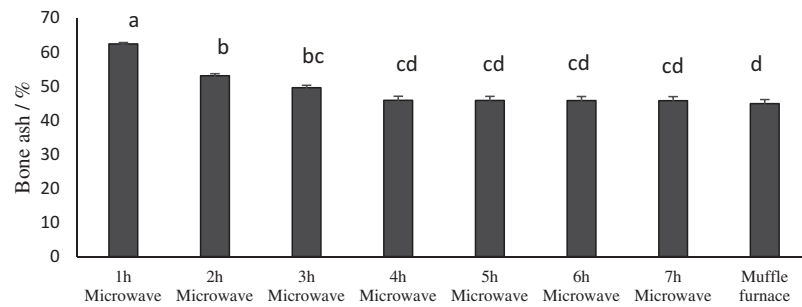


Fig. Comparison of bone ash content (%) for various microwave ashing systems (MAS) and muffle furnace.

RESULTS

Bone ash content was found to be significantly different after 1, 2 and 3 h of MAS ashing compared 4, 5, 6 or 7 h of MAS ashing or MF ashing. No significant difference ($P = 0.846$) was found between the ash percentage after 4 h in the MAS and 13 h in the conventional MF (Fig.).

MF. While the MAS method is useful in terms of decreased time to perform single determinations, the increased cost and decreased capacity of the microwave system make this method a less attractive option for high throughput of bone ash determination in poultry.

ACKNOWLEDGEMENT

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CONCLUSION

Four hours is required to completely ash bones in the microwave system rather than 1-h manufacturer's estimate. This is possibly due to the dryness of the bones, which is unavoidable when expressing ash as a proportion of dry bone weight. No increase in throughput is achieved through using MAS as only three runs (24 tibias) can be processed per day, compared to 23 tibias in a conventional

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Influence of *in ovo* administration of organic zinc on performance and tibia characteristics of broilers

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APPLICATION

In ovo administration or dietary supplementation of organic or mineral sources of zinc can have a beneficial effect on chick weight and hatchability, broiler performance and morphometric characteristics of tibias.

of organic zinc on performance and tibia characteristics of broilers.

MATERIALS AND METHODS

A total of 240 fertile eggs from Ross breeder 308 at day 17E were randomly divided to six treatments as follows: negative control; positive control (phosphate buffered saline (PBS)); eggs injected (once at extra-embryonic cavity) by Zn-Met or ZnSO₄ (0.075 g/l); or dietary supplemented by Zn-Met or ZnSO₄ (40 mg/kg) to the chicks. Diet used in this experiment was based on Ross 308 recommendation for positive control group and the same for others without zinc supplementation in mineral mix chicks were raised to 21 days post-hatch. There were 4 replicates of 10 birds. Diets were offered *ad libitum* throughout the experiment. Diet content average feed intake and average weight gain were measured and FCR was calculated. The right tibias of 4 birds from each replicate were used to measure bone characteristics such as bone weight, bone length, Seedor index, bone breaking and bone ash. The experiment was approved by the Animal Ethics Committee of the Tarbiat Modares University. Data were analysed using the general

INTRODUCTION

For embryos and hatchling chicks to perform well, it is necessary for broiler breeders to transfer enough nutrition to the embryo and or for it to be supplemented after hatching. Zinc is an essential element for many metabolic processes in living organisms. Studies have clearly demonstrated the importance and requirements for zinc in chick embryonic development (Hudson *et al.* 2005). The main sources of minerals used in animal feed are mineral salts, which have low bioavailability. However, organic mineral sources such as proteinate have been used increasingly (Zhao *et al.* 2014). *In ovo* feeding is used as a means to feed embryo prior to its hatching. This experiment was conducted to evaluate the effect of *in ovo* administration

Table. *Effects of different sources of Zn and feeding method on performance and bone characteristics of broiler chicken*

Treatment	Chick weight (g)	Feed intake (g)	Feed conversion	Bone weight (g)	Bone length (mm)	Seedor index (mg/mm)	Bone break resistance (kg)	Bone ash (%)
Positive control	728.7	1020	1.49	11.12 ^c	80.21	136.4 ^c	71.72 ^c	42.88 ^c
Negative control	722.3	1011	1.49	11.19 ^c	81.21	136.3 ^c	72.59 ^c	43.09 ^b
In ovo ZnSO ₄	750.1	1032	1.46	11.30 ^c	81.32	137.5 ^{bc}	72.30 ^c	43.28 ^a
Fed ZnSO ₄	749.0	1039	1.46	11.39 ^{ab}	81.66	139.3 ^{ab}	73.52 ^b	43.33 ^a
In ovo Zn Meth	746.9	1028	1.46	11.33 ^{bc}	80.91	137.8 ^{bc}	72.66 ^c	43.16 ^a
Fed Zn meth	755.1	1031	1.42	11.57 ^a	81.17	140.5 ^a	74.30 ^a	43.57 ^a
SEM	4.89	3.66	0.44	0.39	0.070	0.414	0.181	0.61
P-value	0.294	0.331	0.0462	0.0008	0.1907	0.0055	<0.0001	0.0057

Values with different superscript **alphabets** are significant ($P > 0.05$). SEM = standard error of the mean.

linear model procedure of SAS appropriate for a completely randomised design. Treatment means were compared using the Duncan's Multiple Range Test, and values were considered statistically different at $P < 0.05$.

bone breaking resistance was improved only by Zn-Met feeding ($P < 0.05$). Ash content of bone was also increased with zinc supplementation.

RESULTS

In ovo injection of either type of zinc source had no significant effects on chicks' weight at hatch and hatchability ($P > 0.05$). Also, **BWG** and feed intake of chicks were not affected, although the lowest and highest **FCRs** were observed for the groups supplemented with Zn-Met and the control ($P < 0.05$). Although bone length was not influenced by treatment, bone weight was improved with zinc consumption. Feeding of either Zn source, especially Zn-Met, led to an increase in the Seedor index of the tibias when compared to other groups ($P < 0.01$). At day 21,

CONCLUSION

Zinc supplemented diets, especially organic zinc sources, can improve broilers performance and skeletal characteristics. However, the feeding Zn is more effective than its injection.

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The effect of glycosylation on antimicrobial activity of the egg cuticle

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APPLICATION

Transmission of pathogenic microorganisms from the environment into the egg leads to reduced embryo viability and loss of egg production and creates a public health risk. The cuticle is a glycosylated proteinaceous layer, which contributes to defence against pathogen ingress, understanding its activity is important for improving egg quality. Previous studies have examined the effect of presence or absence of the cuticle on penetration of microorganisms and show that cuticle influences microorganism penetration, but whether the degree of glycosylation plays a role in microorganism penetration and cuticle function is yet to be examined.

INTRODUCTION

The cuticle is a glycosylated proteinaceous layer, which contributes to defence against pathogen ingress via trans-

shell penetration of the egg. The cuticle is comprised of several proteins, the majority of which are heavily glycosylated, including ovocalyxin-36, kunitz like protease inhibitor, ovocleidin-116, ovocleidin-17, ovocalyxin 25, clusterin and ovocalyxin-32 (Bain, *et al.* 2013). Cuticle creates an effective barrier preventing the movement of water across the shell by plugging the gaseous exchange pores, reducing the opportunity for bacterial contamination of the egg contents and through direct antimicrobial action. Protein glycosylation may play a role in cuticle function as it allows for structural stability of glycoproteins, and glycoprotein substrate adhesion. Glycosylation of proteins is essential for multiple biological processes, as such, deglycosylation may impact the structure and function of proteins increasing the risk of pathogen invasion. Previous studies have examined the effect of presence or absence of the cuticle and show that cuticle influences microorganism penetration, but whether the degree of glycosylation plays a role in microorganism penetration is yet to be examined.

MATERIALS AND METHODS

Briefly, cuticle was stripped from the egg using 5% EDTA and deglycosylated either in the native form or after protein denaturation using New England BioLabs Protein Deglycosylation Mix. After deglycosylation, gel purification was used to remove residual enzyme and separate proteins into fractions of <30 kDa and >30 kDa. Non-deglycosylated cuticle proteins were used as a positive control. Protein concentration was normalised between groups. A broth-based antimicrobial assay was used to determine the efficacy of the cuticle proteins against a gram-negative bacteria, *E. coli* DH5a, and a gram-positive bacteria, *S. aureus*. Results are expressed as a reduction in CFU/mL. In order to determine if deglycosylation of proteins was important to antimicrobial function *in situ*, a deglycosylation mix was prepared as described above and pipetted directly onto the eggshell, covered with a parafilm square, and incubated at 37°C for 4 h. In order to visualise if deglycosylation was successful and that cuticle coverage was still intact, two dyes were used: a cuticle dye (Tartrazine/Lissamine Green) and a glycostain (alcian blue in glacial acetic acid, pH 2.5). Buffer mix containing no enzyme was used as a negative control on the same egg. ANOVA was carried out and significance between groups was determined by least significance difference.

RESULTS

Proteins from the cuticle possess antibacterial activity against both gram-negative and gram-positive organisms. The larger cuticle proteins (>30 kDa) were the most efficacious, achieving a significant 95% reduction in *E. coli* ($P < 0.001$ compared to <30kDa) and a 97% reduction in *S. aureus* ($P < 0.001$ compared to <30kDa). This activity was not reduced when the proteins were deglycosylated; however, when the proteins were denatured before being deglycosylated, there was a reduction in the ability to kill *E. coli*. The smaller fraction of cuticle proteins (<30 kDa)

showed no activity against *E. coli* and moderate activity (46%) against *S. aureus*, this increased to 75% when deglycosylated and was not affected by denaturation. The incubation of eggs with deglycosylation enzymes appeared to result in the removal of cuticle, so it was not possible to determine the effect of deglycosylation on the ability of eggs to prevent bacterial penetration using this method. It appeared that the alcian blue stained more than just the glycosylated proteins in the cuticle, probably staining deeper into the shell layer, therefore an alternative method of confirming deglycosylation would need to be investigated.

CONCLUSION

This study confirmed that the cuticle is antimicrobial, but deglycosylation did not appear to have an effect on antimicrobial activity except in the <30 kDa cuticle fraction against *S. aureus* where it improved activity. However, glycosylation may still be an important feature in bacterial defence *in situ* as it is known to provide structural stability.

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Effect of black seed (*Nigella sativa*) and garlic dietary inclusion on carcass characteristics and cellular immunity of broilers

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APPLICATION

Irrational use of antibiotics is a great global health concern in poultry industry. Use of antibiotics as growth promoters in animal feeds has been banned in Europe since 2006. Accordingly, poultry producers seek to find alternative feed additives to improve their flock's productivity.

immunity and productive performance (Garba *et al.* 2013; Umar *et al.* 2015). A lot of work was conducted to investigate their effects on growth performance and blood parameters. However, there have been a limited number of studies associated with the immunomodulatory effects of black seeds and garlic in chickens.

MATERIALS AND METHODS

A total of 700 one day-old broiler chicks (Ross 308) were grown over a period of 42 days. Chicks were wing-banded, weighed individually and randomly divided into nine different dietary treatments. Black seeds (*Nigella sativa*), as a

INTRODUCTION

Black seeds (BS) and garlic (G) are frequently used in broiler as antimicrobial feed additives to enhance

Table. Effect of black seed and garlic dietary supplementation on carcass traits and cellular immune response of broilers.

Item	Cont	Black seed			Garlic			SEM	P-value
		1%	2%	3%	1%	2%	3%		
Body weight at 6 weeks, g	1968.2	1934.5	2090.0	1925.8	1981.6	1877.3	1976.2	31.5	NS
Dressed carcass, %	71.7	71.5	72.7	72.5	72.7	71.4	70.8	0.28	NS
Thigh, %	9.1	9.9	9.0	9.1	9.3	9.9	9.0	0.19	NS
Drum stick, %	4.8	4.9	4.8	4.9	5.1	4.9	4.9	0.04	NS
Breast muscles, %	10.5	10.1	9.9	10.4	10.0	10.6	10.2	0.13	NS
Toe-web swelling after 24 h, mm	0.51 ^c	0.61 ^b	0.58 ^{bc}	0.56 ^{bc}	0.57 ^{bc}	0.63 ^b	0.72 ^a	0.03	0.05
Toe-web swelling after 48 h, mm	0.34 ^b	0.48 ^a	0.37 ^b	0.35 ^b	0.33 ^b	0.38 ^b	0.49 ^a	0.03	0.05
Toe-web swelling after 72 h, mm	0.20	0.29	0.20	0.18	0.22	0.19	0.23	0.02	NS

Means within rows with no common superscript alphabets are significantly differed. NS: non-significant.

whole seed, and powder garlic were added to diet in different levels (0, 1% BS, 2% BS, 3% BS, 1% G, 2% G, 3% G). Each dietary group has 5 replicates (20 chicks each). Feed and water were supplied *AL*. All birds were raised under similar husbandry and environmental conditions. To examine the cell-mediated immune response, 8 chicks from each dietary group at 3 weeks of age were randomly assigned. Each chick was intradermally injected in the toe-web (between the second and the third digit) of the left foot with 100 µg phytohemagglutinin-P (PHA-P) (Sigma Chemical Co., St.Louis, MO 63 178) in 0.1 mL sterile saline. The control toe web of the right foot received 0.1 mL of sterile saline in an identical manner. The thickness of toe-webs was measured with a constant tension calliper before injection and at 24, 48 and 72 h after PHAP injection. The toe-web swelling was calculated as the difference between the thickness of the toe web before and after injection. At 6 weeks of age, 10 chicks/dietary group were randomly chosen to evaluate carcass characteristics. Dressed carcass, thigh, drum stick and breast muscles were weighed and determined relative to live *BW*. The care and handling of birds are in accordance with the regulations of animal care committee of Qassim University. Data was subjected to a one-way *ANOVA* using SAS software.

RESULTS

As shown in the Table, there were no significant differences among dietary groups for *BW* or carcass characteristics. However, a numerical increase in dressed carcass was noticed in broilers that fed a diet containing 2% and 3% BS or G1%. It is of interest to note that supplementation diet with 2% and 3% garlic or 1% black seeds significantly ($P < 0.05$) enhanced cellular immunity of the broilers at the earlier period after PHA-P injection (24 and 48 h post-injection). This improvement went down at the later period.

CONCLUSION

We conclude that the inclusion of black seeds or garlic in broiler's diet greatly enhanced cell-mediated immune response, without penalising carcass quality.

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Effect of naked neck and frizzle genes on cell-mediated immunity, concentration of serum immunoglobulins and lymphoid organs of chickens

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APPLICATION

The introduction of major genes, particularly naked neck (Na) and frizzle (F) genes in chickens raised under hot environmental conditions, may improve productive performance and immunocompetence.

INTRODUCTION

As the rural sector of poultry continues to expand in the Kingdom of Saudi Arabia, breeds resistant to heat stress

has become a major concern in many hot regions. The prestigious major genes affecting feather coverage of chicken, particularly naked neck (Na) and frizzle (F), are well known for the effects on heat tolerance and superior productive performance under high environmental conditions (Mahrous *et al.* 2008; Fathi *et al.* 2013 and Fathi *et al.* 2014). These major genes are also believed to confer resistance to diseases and greatly enhance immune status. Therefore, a study was conducted to evaluate their potential effects on immune profile in Saudi native breeds carrying them in homozygous manner.

Table. *Effect of naked neck and frizzle genes on immune response parameters of chickens*

Item	Genotype			SEM	P-value
	NaNaff	NaNaffF	NaNafff		
Swelling response after 24 h, mm	0.66 ^a	0.51 ^a	0.28 ^b	0.06	0.001
Swelling response after 48 h, mm	0.29	0.31	0.21	0.03	NS
Swelling response after 72 h, mm	0.23 ^a	0.25 ^a	0.12 ^b	0.03	0.01
Serum IgA, µg/mL	0.45	0.40	0.47	0.02	NS
Serum IgM, µg/mL	3.09	3.23	2.82	0.13	NS
Serum IgY, µg/mL	5.51	5.42	5.45	0.13	NS
Thymus, %	0.28	0.27	0.26	0.02	NS
Spleen, %	0.23	0.20	0.22	0.01	NS
Bursa of Fabricius, %	0.11 ^a	0.09 ^{ab}	0.07 ^b	0.01	0.01

Means within rows with no common superscript alphabets are significantly different.

MATERIALS AND METHODS

A total of 197 chickens including 3 genotypes (42 homozygous naked neck (NaNa), 35 homozygous frizzled (FF) and 120 normally feathered (nana)) produced from the same origin were used to evaluate cell-mediated immunity, serum immunoglobulins and relative weight of lymphoid organs. All birds were kept under identical environmental, nutritional and health conditions. To determine the relative weight of lymphoid organs, 10 birds from each genotype were randomly assigned and slaughtered at 8 weeks of age. Serum IgA, IgM and IgY concentrations were determined in appropriately diluted samples by a sandwich ELISA using microtiter plates and chicken-specific quantitative ELISA kits (GenWay Biotech, Inc., San Diego, CA). To examine the cell-mediated immune response, 24 chickens (8 each) aged 33 weeks were used. Each chicken was intradermally injected in the right wattle with 100 µg PHAP (Sigma Chemical Co., St. Louis, MO 63178) in 0.1 mL sterile saline. The thickness of resulting swollen was measured with a constant tension calliper before injection and at 24, 48 and 72 h after PHAP injection. The wattle swelling response was calculated as the difference between its thickness before and after injection. The care and handling of birds are in accordance with the regulations of animal care committee of Qassim University. Data of individual records for genotypes were subjected to a one-way ANOVA using SAS software.

RESULTS

As shown in the Table, a significant improvement ($P < 0.01$) in immune response of cutaneous basophil

hypersensitivity was noticed after 24 and 72 h after injection in naked neck and frizzled genotypes compared with normal feathered genotype. No significant difference was noticed due to genotype for different types of serum immunoglobulins. However, a numerical increase associated with Na and F genes was detected. In terms of relative weight of lymphoid organs, it is of interest to note that the naked neck gene significantly increased bursa of Fabricius compared with normal feathered one, while frizzle was intermediate. Thymus % and spleen % didn't exhibit a significant difference due to genotype.

CONCLUSION

In conclusion, these results indicate that naked neck and frizzle genes significantly improved cell-mediated immunity 24 and 72 h post-injection. While no significant differences among different genotypes of serum immunoglobulins under hot environmental conditions.

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Impact of overnight storage conditions on lactic acid bacteria colony counts in chicken caecal samples

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APPLICATION

Twelve-h storage at 4°C of post-mortem samples of chicken caeca had no significant impact on subsequent lactic acid

bacteria counts. This increases viability of including microbial cultures within poultry studies obviating the need for same-day culturing consequently allowing more time for other time-critical measures on post-mortem collection days.

INTRODUCTION

In recent years, the study of the microbiome and its role in the maintenance of health and development of disease has received increasing research interest. In particular, lactic acid bacteria (LAB) are considered to play an important role in promoting beneficial gut health (Walter 2008). However, during animal trials, several constraints may delay the processing of gut samples for microbial culturing which may impact on bacterial survival. The aim of this study was to investigate the effects of storing chicken caecal samples overnight on LAB levels and to examine the effect of dilution buffer on the recovery of LAB.

MATERIALS AND METHODS

Whole caeca were collected post-mortem from 8 broiler birds aged 21 days in 20-mL universal sterile containers. Samples were immediately stored in cooled boxes with frozen ice packs for short transport (2 h). Samples were serially diluted in maximum recovery diluent (MRD) or (PBS), plated on de Man Rogosa Sharpe agar and incubated (37°C, 48 h, microaerophilic conditions) on the same day of collection and after storage at 4°C for 12 h. After incubation, LAB CFU per g was determined and logarithmically transformed for statistical analysis using general linear model (GLM) with time and buffer as treatment factors in IBM SPSS v.22. Significant differences were considered at $P \leq 0.05$.

RESULTS

Effect of buffer and delayed culturing overnight on LAB counts is shown in the Table. There were no significant effects of buffer or time on LAB count ($P > 0.05$).

Carbohydrases and prebiotics influenced nutrient retention without affecting growth of broilers

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APPLICATION

Carbohydrases are used to combat the negative effects of NSP but they also impact positively on nutrient retention even in diets that already meet nutrient requirement of broiler chickens.

INTRODUCTION

NSP are associated with a decrease in nutrient absorption primarily due to an increase in digesta viscosity (Smits and Annison, 1996) and can also affect nutrient retention. Carbohydrases are added to poultry diets to improve growth performance and increase nutrient utilisation (Olukosi *et al.* 2007). This study investigated the addition of carbohydrases or prebiotic oligosaccharides to wheat- or barley-based diets using growth performance and nutrient retention.

Table. Effect of overnight storage at 4°C and dilution buffer on log mean CFU/g of lactic acid bacteria of chicken caecal samples (N = 8) at maximum recovery diluent (MRD) and phosphate buffer saline (PBS).

Sample no.	Collection day		Overnight	
	MRD	PBS	MRD	PBS
1	5.90	5.95	7.07	7.06
2	6.17	7.19	6.25	7.22
3	6.72	6.77	7.30	7.43
4	6.79	6.66	7.46	7.43
5	5.86	6.01	6.86	6.95
6	5.90	5.88	6.95	7.04
7	6.23	6.16	7.17	7.12
8	6.07	6.	7.04	7.12
SEM	0.13	0.11	0.06	0.06

CONCLUSION

The results indicate that chicken caecal samples can be stored at 4°C overnight until the day after post-mortem collection with no significant effect on LAB counts. In addition, both dilution buffer tested showed similar recovery of LAB levels. While this study provides effective guidance on the culturing of LAB from chicken caeca, further work is required to investigate the stability and optimum storage conditions of different sample types for various bacterial population and specific downstream applications.

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MATERIALS AND METHODS

On day zero, 384 Ross 308 broiler chicks were weighed and allocated to one of 8 treatments in a 2 × 4 factorial arrangement. Each treatment had 6 replicates with 8 birds per replicate. The factors were diet type (wheat or barley based) with 4 additives for each diet type (no additive, carbohydrases at 16,000 or 32,000 units/kg or prebiotic oligosaccharide). Wheat-based diets were supplemented with xylanase or xylo-oligosaccharide (XOS) at 0.2 g/kg, whereas barley-based diets were supplemented with β-glucanase or galacto-oligosaccharide (GOS) at 5 g/kg. The diets were formulated to meet the energy requirement of 12.6 MJ/kg and 230 g/kg crude protein and Ti was added as a digestibility marker. The experiment was reviewed by the animal ethics committee prior to commencing. Feed and birds were weighed at the beginning and end of the study and excreta were collected on days 20 and 21. The data on growth performance and nutrient retention were

Table. The impact of diet or additive type on growth performance and coefficients of total tract nutrient retention.

	Wheat				Barley				SEM	P-values		
	CTR	XYL16	XYL32	XOS	CTR	BG16	BG32	GOS		DT	AT	DT× AT
WG, g	771.2	805.6	791.4	801.4	755.2	835.9	792.6	745.7	40.5	NS	NS	NS
FCR	1.53	1.41	1.45	1.36	1.45	1.38	1.45	1.51	0.069	NS	NS	NS
DM	0.66 ^b	0.71 ^b	0.73 ^c	0.69 ^b	0.67 ^b	0.60 ^a	0.66 ^b	0.72 ^c	0.016	0.007	0.010	<0.001
AME	13.6 ^a	15.9 ^b	14.3 ^b	14.4 ^b	14.3 ^b	14.6 ^b	14.7 ^b	12.7 ^a	0.145	<0.001	<0.001	<0.001
N	0.65	0.66	0.71	0.66	0.69	0.60	0.69	0.67	0.019	NS	0.013	NS

Means within a row but with different superscript alphabets are significantly different ($P < 0.05$). Comparisons were made within groups namely either wheat group or barley group. CTR: control; XYL16 and XYL 32: xylanase at 16,000 or 32,000 units/kg; BG16 and BG32: β -glucanase at 16,000 and 32,000 units/kg; XOS: xylo-oligosaccharides; GOS: galacto-oligosaccharides; DT: diet type; AT: additive type; WG: body weight gain; FCR: feed conversion ratio; DM: dry matter; AME: metabolisable energy (MJ/kg); N: nitrogen retention; SEM: standard error of the means, NS: non-significant.

2735 analysed using the general ANOVA function of Genstat and significance was declared at $P \leq 0.05$.

RESULTS

2740 There was no significant treatment effect for BW gain or FCR. There was a diet type \times additive type interaction for total tract DM retention and AME. In wheat diets, DM was significantly higher ($P < 0.001$) than control when 32,000 U/kg of xylanase was supplemented, whereas in barley diets, DM was significantly lower than the control ($P < 0.001$) when 16,000 U was added but significantly increased ($P < 0.001$) when GOS was added. AME in wheat diets was significantly greater ($P < 0.001$) than the control when either xylanase or XOS was supplemented; however, there was no effect of β -glucanase supplementation in barley diets but there was significant decrease ($P < 0.001$) in AME in diet in which GOS was supplemented. There was significant main effect of additive on N retention ($P < 0.05$). N retention was lower, relative to the control, when enzyme was supplemented at 16,000 units/kg but N retention increased ($P < 0.05$) in diets supplemented with 32,000 units/kg.

CONCLUSION

Supplementation of nutrient adequate diets with carbohydrases or prebiotic oligosaccharides could alter nutrient retention without having any apparent effect on the growth performance of birds. This demonstrates a possible disconnect between the effect of additives on growth performance and the effect on nutrient retention in certain scenarios and shows that growth performance alone may not be the sole criterion for assessing efficacy of additives used in broiler diets.

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The effect of xylanase supplementation to diets containing wheat ddgs on laying hen performance

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APPLICATION

2780 Supplementation of xylanase to laying hen diets containing wheat DDGS improves energy availability.

INTRODUCTION

2785 In the UK, distillers dried grains with solubles (DDGS) is primarily produced using wheat. The removal of starch during ethanol production results in residual components such as fibre, protein and fat becoming up to three times

more concentrated. NSP are the predominant constituents of fibre. When fed to poultry, they have been found to possess anti-nutritional effects as birds lack the endogenous enzymes required to hydrolyse the NSP content of feeds (Choct 2006). Xylanase is used within monogastric diets to counteract the adverse effects caused by anti-nutritional factors such as NSP. However, little research is available on the effect of exogenous xylanase on wheat by-products that have undergone fermentation (Widyaratne *et al.* 2009). The aim of this experiment was to investigate the effect of a high level of wheat DDGS with and without the addition of xylanase when supplemented into laying hen diets.

Table. The effect of wheat distillers dried grains with soluble (DDGS) and xylanase on feed intake (FI), body weight gain (g/b/d), egg production (%), egg mass (g/b/d), feed conversion ratio (FCR) for egg production and apparent metabolisable energy (AME) from 23 to 43 weeks of age.

	Feed intake (g/b/d DM)	Body weight gain (g/b/d)	Egg production (%)	Egg mass (g/b/d)	FCR for egg production	AME (MJ/kg DM)
DDGS						
–	107.0	2.71	92.3	59.0	1.932	12.70
+	98.8	1.26	85.0	50.1	2.093	12.90
Xylanase						
–	103.1	1.84	86.9	53.7	2.035	12.63
+	103.8	2.12	90.5	55.4	1.990	12.97
SEM	1.160	0.097	0.009	0.71	0.022	0.107
DDGS × xylanase						
– DDGS – xylanase	107.5	2.67	90.6	58.1	1.951	12.79 ^a
– DDGS + xylanase	108.7	2.75	93.9	60.0	1.914	12.62 ^a
+DDGS – xylanase	98.7	1.02	83.2	49.4	2.110	12.48 ^a
+ DDGS + xylanase	99.0	1.50	87.1	50.9	2.066	13.31 ^b
SEM	1.640	0.136	0.013	1.00	0.031	0.151
Probabilities of statistical differences						
DDGS	<0.001	<0.001	<0.001	<0.001	<0.001	NS
Xylanase	NS	0.044	0.052	0.093	NS	0.038
DDGS × xylanase	NS	NS	NS	NS	NS	0.003

MATERIALS AND METHODS

A wheat-soybean meal control diet was prepared, containing 11.60 MJ/kg AME and 166.5 g/kg crude protein. A second wheat-soybean meal-based diet was also prepared to contain 300 g/kg wheat DDGS. The DDGS diet was formulated to have the same AME as the control diet and a crude protein content of 171.1 g/kg. The two diets were divided into two equal parts and half of them were supplemented with 2500 U/kg of Danisco xylanase resulting in 4 diets in total (20 reps). Diets were fed *AL* to 320 Hy-Line Brown laying hens from 23 to 43 weeks of age. Egg numbers were recorded daily, while egg weights were recorded weekly. Dietary AME was determined using the total collection technique. Data were statistically analysed by ANOVA using a 2 × 2 factorial arrangement of treatments. Duncan's multiple range test was used to determine significant differences between treatment groups. Differences are reported as significant at *P* < 0.05.

RESULTS

The main effects were the inclusion of wheat DDGS and the addition of xylanase (Table). The inclusion of wheat

DDGS reduced feed intake, BW gain, egg production and egg mass and increased FCR for egg production. An interaction was observed between DDGS and xylanase for AME. The interaction shows diets containing wheat DDGS that were supplemented with xylanase-improved AME.

CONCLUSION

Feeding a high level of wheat DDGS caused production to be much lower than that of the breed standards. It is likely that the reduction in feed intake negatively affected BW gain, egg production, egg mass, and FCR for laying hens. The addition of xylanase increased bird weight and tended to improve egg production and egg mass. The DDGS and xylanase interactions for AME show that this enzyme improved energy utilisation in diets containing wheat DDGS.

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The mycotoxin profile of wheat and its relationship with broiler performance

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APPLICATION

Broiler performance is affected by wheat variability but the relationship between mycotoxin content and performance is weak and variable.

INTRODUCTION

Mycotoxins are toxic chemicals that are produced by fungi which infect crops in the field or during storage. Overall, the potential negative effect of mycotoxin contamination

of broiler feed has been well documented. However, in practice, their actual effect on broiler performance has not been well established (D'Mello and Macdonald 1997) and the majority of work has been conducted on the most common wheat mycotoxins, deoxynivalenol and zearalenone. There is a lack of information on the entire mycotoxin profile of wheat and how that relates to broiler performance. The aim of this study was to determine the mycotoxin content profile across a range of wheat samples and to establish the relationship between specific mycotoxins and broiler performance.

MATERIALS AND METHODS

Twenty wheat samples were sourced from across Northern Ireland ($N = 7$), Republic of Ireland ($N = 2$), Great Britain ($N = 8$), USA ($N = 1$), Germany ($N = 1$) and France ($N = 1$). Samples were screened to detect and quantify 79 mycotoxins using QUECHERS extraction method and LC-MS/MS analysis. Starter, grower and finisher broiler diets were formulated using each wheat sample (at least 600 g/kg wheat). The diets were balanced for ME, crude protein and lysine and were formulated to meet standard nutrient specifications (Ross 308). Diets were offered to a total of 1200 male broilers in pens of 10, giving 6 pen replicates/wheat. Dry matter intake (DMI), liveweight gain (LWG) and FCR were determined for the starter (0–14 d), grower (14–21 d), finisher (21–35 d) and overall period (0–35 d). Results were analysed by ANOVA using Genstat to test for the effect of wheat sample. Simple regression analysis was conducted to determine the relationship between mycotoxin content and performance (only ZON and those mycotoxins which were present in over 50% of the wheat samples were correlated with performance parameters).

RESULTS

Thirty nine (out of a total of 79 screened) mycotoxins were detected across the 20 wheat samples. The following were detected with the number of wheat samples they were detected in (out of 20) in brackets: beauvericin (14), citrinin (1), cyclopiazonic acid (1), enniatin A (20),

enniatiin A1 (20), enniatiin B (20), enniatiin B1 (20), ergocornine (3), ergocorninine (1), ergocristine (7), ergocristinine (4), ergocryptine (4), ergocryptinine (4), ergometrine (7), ergometrinine (4), ergosine (3), ergosinine (3), ergotaminine (5), alternariol (2), aurofusarium (20), curvularin (1), don (20), emodin (3), equisetin (16), fumonisin B1 (2), fumonisin B2 (1), meleagrin (11), mycophenolic acid (7), ochratoxin A (3), ochratoxin B (1), penitrem A (2), roquefortine C (3), sterigmatocystin (10), tentoxin (2) and zearalenone (9). The range in the most predominant mycotoxins is shown in the Table. There were significant differences ($P < 0.05$) in broiler performance as a result of wheat sample. Overall DMI, LWG and FCR ranged 3000–3350 g (SEM = 83.5), 2017–2327 g (SEM = 55.4) and 1.41–1.49 (SEM = 0.045), respectively. When performance parameters were correlated with mycotoxin content, most relationships were weak and non-significant. ZON was negatively related to overall DMI ($R^2 = 0.61$ and $P = 0.013$).

CONCLUSION

Broiler performance was significantly influenced by wheat sample which is in keeping with previous research (Ball *et al.* 2013). There was range of mycotoxins detected in the wheat samples indicating that the mycotoxin profile is variable. Wheat ZON content was negatively related to broiler DMI but not to LWG or FCR. Overall, the relationship between wheat mycotoxin content and broiler performance is weak and variable.

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Table. *Wheat mycotoxin profile and the relationship (R^2) between wheat mycotoxin content and broiler performance.*

	Wheat mycotoxin profile (ng/g)				Overall DMI		Overall LWG		Overall FCR	
	Min	Mean	Max	SD	R^2	P-value	R^2	P-value	R^2	P-value
Beauvericin	0.15	1.10	3.49	6.99	0.05	0.218	0.01	0.837	0.01	0.299
Enniatin A	0.55	13.12	59.12	17.59	0.01	0.680	0.12	0.073	0.09	0.111
Enniatin A1	3.73	76.89	303.42	93.55	0.01	0.707	0.07	0.138	0.04	0.194
Enniatin B	34.70	3541.07	12 157.10	4079.72	0.01	0.632	0.09	0.107	0.05	0.168
Enniatin B1	10.68	424.70	1546.20	493.50	0.01	0.709	0.06	0.154	0.04	0.207
Aurofusarin	12.17	308.90	1005.68	307.00	0.03	0.476	0.12	0.073	0.05	0.184
Deoxynivalenol (DON)	22.02	218.02	677.84	187.18	0.03	0.462	0.01	0.410	0.01	0.984
Equisetin	1.54	5.60	23.69	5.45	0.01	0.284	0.01	0.495	0.01	0.569
Zearalenone (ZON)	1.94	11.22	38.40	12.30	0.61	0.013	0.16	0.155	0.01	0.378